The production and synthetic utility of the dioxygenase-derived metabolites of substituted aromatics.

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Abstract

The substrate scope and selectivity of toluene dioxygenase overexpressed in E.coli JM109 (pDTG601A) was investigated with series of *ortho*-halobenzoates and *para*-substituted arenes. Palladium-catalyzed carbonylation methodology was developed to convert halogenated *cis*-dihydrodiol metabolites to the corresponding carboxylates and a comparison of the overall efficiency between the enzymatic and chemical methods of access was made. Some of the metabolites produced by toluene dioxygenase were employed in a synthetic approach toward tetrodotoxin. Enzymatic dihydroxylation of benzoic acid with *R. eutropha* B9 provided the corresponding *ipso*-diol that was used in the first total synthesis of pleiogenone A, a bioactive natural product. Experimental and spectral data are provided for all new compounds.

Acknowledgements

I must express my gratitude to my supervisor, Professor Tomas Hudlicky. I have always believed that nothing is gained by having low standards, and Professor Hudlicky's high standards for work ethic and professionalism have allowed me to learn and achieve more in the laboratory than I ever believed possible. I will carry the lessons I have learned from Professor Hudlicky, inside the laboratory and out, for the rest of my life.

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TABLE OF CONTENTS

List of Tables

List of Schemes

List of Figures

List of Abbreviations

acac	acetylacetonate
AIBN	azobisisobutyronitrile
Asp	aspartic acid
ATP	adenosine triphosphate
BCE	before common era
BINAP	2,2'-bis(diphenylphosphino)-1,1'-binaphthyl
bmim	1-butyl-3-methyl-imidazolium tetrafluoroborate
Bz	benzoyl
BZDO	Benzoate dioxygenase
BZDO-O	Benzoate dioxygenase - oxygenase
BZDO-R	Benzoate dioxygenase - reductase
CSA	camphorsulfonic acid
cyp	cyclopropyl
Cys	cysteine
dba	dibenzylidene acetone
DBU	1,8-diazabicycloundec-7-ene
DCC	*N*,*N*'-dicyclohexylcarbodiimide
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DEAD	diethyl azodicarboxylate
DIBAL-H	diisobutylaluminum hydride
DMAP	4-(dimethylamino)pyridine
DMF	dimethylformamide

DMP	dimethoxypropane
DMSO	dimethylsulfoxide
DPPF	1,1′-bis(diphenylphosphino)ferrocene
EPR	Electron paramagnetic resonance
er	enantiomeric ratio
FAD	Flavin adenine dinucleotide
His	histidine
HMDS	hexamethyldisilazane
IBX	2-iodoxybenzoic acid
IC_{50}	Concentraton required to inhibit a given process by 50%
MS	Mass spectrometry
LAH	lithium aluminum hydride
Ms	methanesulfonyl
NADH	nicotinamide adenine dinucleotide
NBS	*N*-bromosuccinimide
NMI	*N*-methyl imidazole
NMO	*N*-methylmorpholine *N*-oxide
NMP	*N*-methyl-2-pyrrolidone
NMR	nuclear magnetic resonance
NDO	naphthalene dioxygenase
PAD	potassium azodicarboxylate
PCC	pyridinium chlorochromate
Ph	phenyl

TBAF	tetrabutylammonium fluoride
TBDPS	*t*-butyldiphenylsilyl
TBS	*t*-butyldimethylsilyl
TDO	toluene dioxygenase
TDO-F	toluene dioxygenase - ferredoxin
TDO-O	toluene dioxygenase - oxygenase
TDO-R	Toluene dioxygenase - reductase
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TMEDA	*N*,*N*,*N*',*N*'-tetramethylethylenediamine
TMS	trimethylsilyl
TPP	tetraphenylporphyrin
Ts	*p*-toluenesulfonyl

1.0 Introduction

In reviewing the accomplishments in the field of organic synthesis since its inception with the synthesis of urea by Wöhler in 1828,[1] some have described the field as a "mature" science.[2] The implication being that organic synthesis is in a state of declining production and that truly revolutionary advances are not likely to be made. Although this idea can readily be refuted when one considers the innovative synthetic approaches which have recently been applied to the production of valuable compounds by Baran[3] and many others, it was most eloquently addressed by Seebach some 25 years ago.[2] Seebach argues that organic synthesis has not become a "mature" science, but has become increasingly integrated with other disciplines, and indeed with all of the natural sciences.[2] Following Seebach's article, Hudlicky published a discourse on design problems in synthesis.[4] This article served as an introduction to a topical issue of *Chem. Rev*. edited by Wender that presented 24 articles by experts in various disciplines representing the state of the art in synthesis in 1996.[5] The field of organic synthesis cannot be considered a "mature science" because of the continued contributions from other fields, and because of the questions in the field that have yet to be answered.

An example of a discipline which has continually made significant contributions to organic synthesis is the field of biocatalysis.[6] Through the identification of enzymes and the isolation of metabolites, the field of biocatalysis has made the practical synthesis of many important compounds possible.[6] The advances in the field of biocatalysis have also provided the means of meeting the metrics for efficiency in organic synthesis. This is true of traditional metrics such as step economy,[7] atom economy[8] and redox economy,[9] and is particularly true when metrics which take into account environmental factors such

as effective mass yield (mass of product / mass of all non-benign materials used)[10] or E-factor (mass of total waste / mass of product)[11] are applied. Enzymatic transformations are typically performed in aqueous media and byproducts are often entirely biodegradable, therefore biocatalysis significantly increases the efficiency of a synthesis when metrics such as effective mass yield are applied. These advantages have led many synthetic chemists to adopt the use of enzymatic transformations.

A particularly fruitful example has been the use of dioxygenase enzymes such as toluene dioxygenase (TDO)[12] and benzoate dioxygenase (BZDO)[13] to produce *cis*-cyclohexadienediol metabolites, such as **2**[14] and **4**[15] respectively, which have been widely used in enantioselective synthesis (Figure 1).

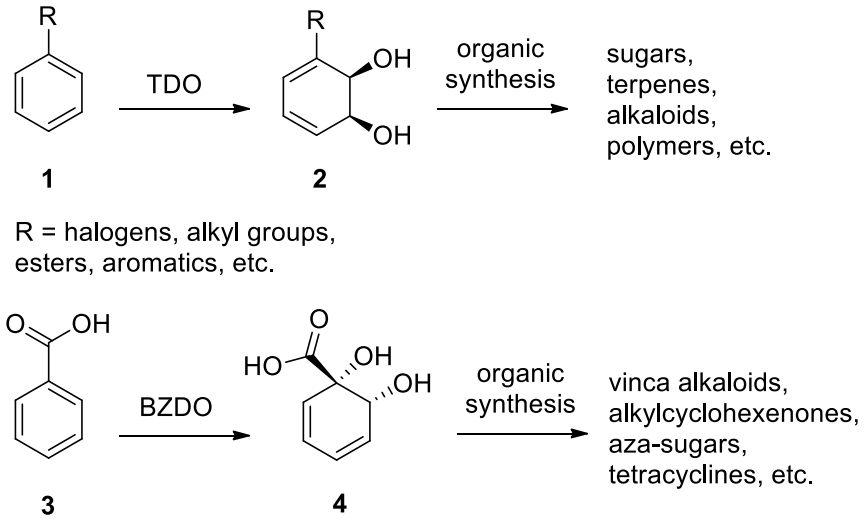
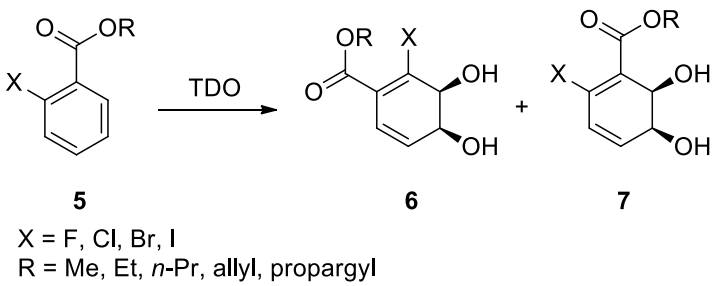


Figure 1: Dihydroxylation of substituted aromatics by toluene dioxygenase (TDO) and benzoate dioxygenase (BZDO) and the application of metabolites in synthesis.[12-15]

This thesis will describe new developments in the utility of these metabolites. First, the study of the substrate scope and selectivity of the enzymatic dihydroxylation

performed by TDO will be undertaken. This study will apply a small library of *ortho*-halobenzoates (**5**) as substrates for the enzyme (Figure 2).

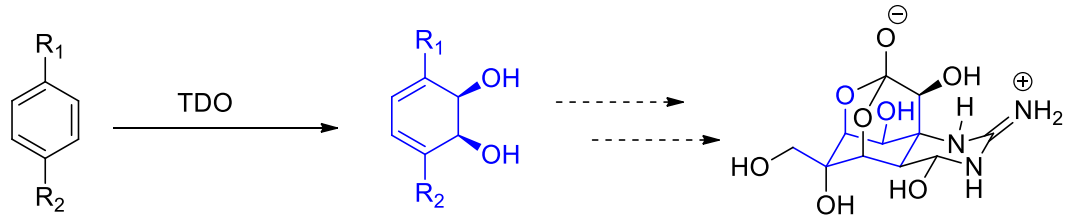


5 **6** **7**

X = F, Cl, Br, I
R = Me, Et, *n*-Pr, allyl, propargyl

Figure 2: The study of the enzymatic dihydroxylation of *ortho*-halobenzoates.

Second, *para*-disubstituted aromatics (**8**) will be investigated as substrates for toluene dioxygenase, with the goal of identifying a suitable starting material for a potential synthesis of tetrodotoxin (**10**) (Figure 3).



8 **9** **tetrodotoxin (10)**

R_1 = CH_2OH, CHO, CH_2OAc, CH_2CHCH_2, CCH, CCHTMS
R_2 = Br, I

Figure 3: Investigation of *p*-disubstituted aromatics as substrates for toluene dioxygenase (TDO).

Third, carbonylation methodology will be explored to provide a more practical route to **(13)** which has been identified as a useful starting material in the synthesis of oseltamivir,[16] as well as other compounds (Figure 4).

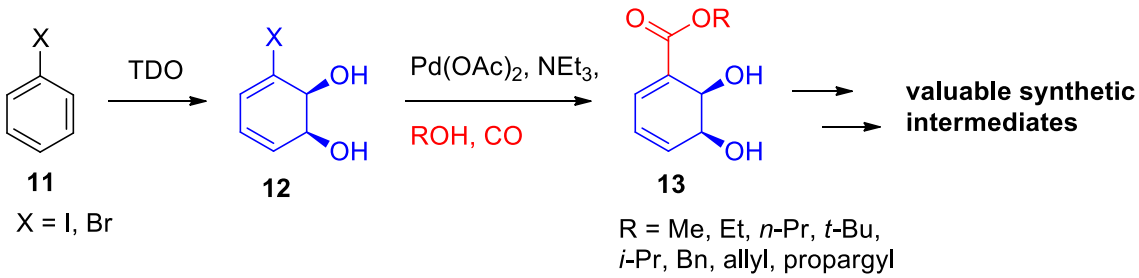


Figure 4: Carbonylation of halogenated metabolites to their corresponding carboxylates.

Finally, the enzymatic metabolite of benzoic acid, *ipso*-diol (**4**), will be used in an approach to the synthesis of a recently identified[17] alkylcyclohexenone natural product, pleiogenone A (**14**) (Figure 5).

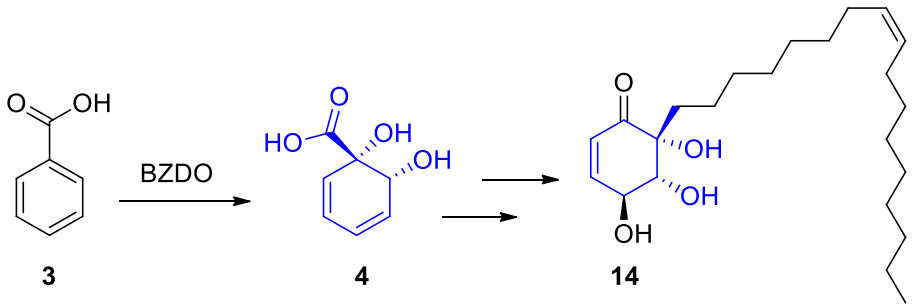


Figure 5: Chemoenzymatic synthesis of pleiogenone A (**14**).

The following section will provide historical background for each of the endeavours described above.

2.0 Historical

The following historical chapter is divided into four sections covering: 1) History of Biocatalysis, 2) Arene dioxygenases, 3) Carbonylation chemistry and 4) Alkylcyclohexenone natural products. The role of arene dioxygenase enzymes in the degradation of environmental contaminants by soil bacteria, as well as the importance of the metabolites produced in organic synthesis will be discussed. A brief discussion of the history and importance of carbonylation chemistry will be provided. The isolation, synthesis and biological significance of alkylcyclohexenone natural products will also be discussed.

2.1.1 History and evolution of biocatalysis

The most ubiquitous example of the use of enzymatic transformations throughout history is their application in the production of fermented beverages. This process can be dated back as far as 7000 BCE, when rice, honey, and fruits were combined to make fermented beverages in China.[18] It is clear that the role of biological entities in the production of these beverages was recognized even at this time. The fermentation process in China has been shown to have evolved over time to include the addition of *Aspergillus*, *Rhizopus* and/or *Monascus* fungi (among others) to promote amylolysis.[18] The production of fermented beverages also has a long history in other countries, including ancient Egypt, where it has been shown that *Saccharomyces cerevisiae* was added to beverages to promote fermentation as early as 3150 BCE.[19] In addition to using biological entities in the production of fermented beverages, it has also been shown that natural extracts were added to prevent the growth of bacteria responsible for their

spoilage.[20] Indeed, the earliest wine vessel ever discovered (5400-5000 BCE, Northern Iran) was shown to contain an extract from the *Pistacia atlantica* tree which prevents the growth of *Actinobacter* responsible for the conversion of ethanol to acetic acid.[20] Whether or not the exact nature of the biological entities being employed was understood, it is clear that humans have had a long history of utilizing the power of enzymatic transformations to their benefit.

Despite thousands of years of experience, it was not known whether fermentation was a biological or purely chemical process. In 1833, Payen and Persoz made a tremendous step in the understanding of enzymatic catalysis.[21] Payen and Persoz isolated a white precipitate from barley malt extract, which was shown to catalyze the conversion of starch to maltose, making this the first *in vitro* enzymatic transformation.[21] Payne and Persoz named the white precipitate diastase, thereby establishing the convention of naming enzymes with the suffix "-ase".[21] In the study of the fermentation process, it was still not known whether yeast was a living organism or a purely chemical entity. van Leeuwenhoek observed yeast cells as early as 1680, but did not regard them as living organisms.[22] In the late 1830s, Caginard de Latour, Kutzing and Schwann all independently determined that yeast was indeed a living organism, having observed yeast cells reproducing through budding.[23] Schwann would go on to refer to yeast as "sugar-fungus" and Kuntzig proposed that yeast was an organized body and not an individual compound.[23] With yeast identified as a living organism, the understanding of enzymatic transformations was taken a step further by Traube in 1858.[23] Traube proposed that all fermentation processes occuring in living organisms were caused by discrete chemical entities called "ferments" that were produced in the cell. Furthermore, Traube considered

that these "ferments" were closely related to proteins and that their function was to transfer oxygen and hydrogen molecules to facilitate intramolecular oxidation and reduction.[23] Traube's ideas about fermentation and the biological entities that cause this process, began to approach the modern understanding of enzymatic transformations.

The final proof that fermentation is indeed a biological process was provided by Pasteur in 1857. Pasteur meticulously demonstrated that the products of alcoholic fermentation were more numerous and complex than just ethanol and carbon dioxide, indicating that a portion of the fermentable material was being integrated into the yeast. Furthermore, Pasteur demonstrated that alcohol production occurs simultaneously with yeast multiplication, and that the yeast must be alive for fermentation to occur.[24,25] In addition to these seminal contributions, Pasteur was also the first to observe the stereospecificity of enzymatic transformations. He reported a "mode of fermentation" of tartaric acid, which occurred very readily with one enantiomer of tartaric acid and not at all with the opposite enantiomer.[24] Pasteur had settled the debate about the biological nature of fermentation, and provided tremendous insight on the "ferments" which had been proposed by Traube.[23] Just twenty years after Pasteur's seminal report, Kuhne renamed these "ferments" enzymes, as it was recognized that an intact organism was not required for enzyme activity to occur.[26]

With the increased understanding of the biological nature of fermentation, chemists began to recognize the potential to harness these biotransformations, leading to the birth of the field of biocatalysis. The true inception of the field of biocatalysis occurred in 1886, when Brown used *Actinobacter* cells to perform a series of biotransformations including the conversion of ethanol to acetic acid and the conversion

of mannitol to fructose.[27] Brown recognized the value of this work to the field of chemistry, as the following quote demonstrates:

"*I think the experiments just described will be of interest to biologists as well as chemists, as they help to show that the vital functions of certain organized ferments are most intimately connected with the molecular constitution of bodies on which they act*."[27]

The field of biocatalysis was expanded from the use of whole cells to the use of enzyme extracts in 1903, when Dakin utilized crude pig liver esterase to perform the kinetic resolution of ethyl mandelate.[28] This seminal report led to the widespread application of lipases as catalysts for acylation, ester hydrolysis, and as a means for desymmetrization and resolution.[6,29] The first report of the use of yeast to perform the reduction of a carbonyl was made in 1911 by Lintner and von Liebig.[30] This finding represents one example of the advances in biocatalysis directly benefitting synthetic chemists.

In 1894, Emil Fischer provided a significant advance in the understanding of enzymatic reactions by proposing the "lock and key" model for the interaction of an enzyme and its corresponding substrate.[31] This model describes the specific interactions between an enzyme and a substrate, and effectively explains the stereospecificity that was observed by Pasteur in his study of fermentation 40 years earlier. Fischer's "lock and key" model has evolved over time to include the concept of "induced fit", which was introduced in 1958 by Koshland.[32] The "induced fit" model describes the conformational changes that occur to an enzyme upon binding a substrate, caused primarily by non-covalent interactions between the substrate and amino-acids in the active site. These conformational changes serve to orient the substrate and the amino-acids in the active site

so as to stabilize the transition state of the reaction in question.[32] The induced fit model, proposed 100 years after Traube first hypothesized the existence of "ferments", is represents just one of the tremendous gains that have been made over this period of time.

The role of enzymes as biological catalysts that increase the rate of reactions by decreasing the activation energy, was well understood by the early 1900s.[33] This allowed biochemists to envision many practical applications for enzymes. As early as 1908, Rosenberg utilized nitrilase enzymes isolated from almonds in the synthesis of optically active cyanohydrins.[34] In 1916 it was shown that enzymes could be immobilized on solid supports without a loss of activity.[35] Enzymes began to be employed in new industrial applications including the introduction of pancreatic enzymes for the cleaning of laundry in 1908.[33] This trend continued until the cultivation of the first industrial scale cultures of *Bacillus licheniformus* for the production of protease enzymes in 1960.[33]

Today, the advent of technologies such as selective mutagenesis and directed evolution have allowed for the design of catalysts for specific transformations or to operate under a particular set of desired conditions.[36] The continued use and improvement of these techniques allowed for the development of biocatalysts for organic synthesis beyond what can be isolated from natural sources.

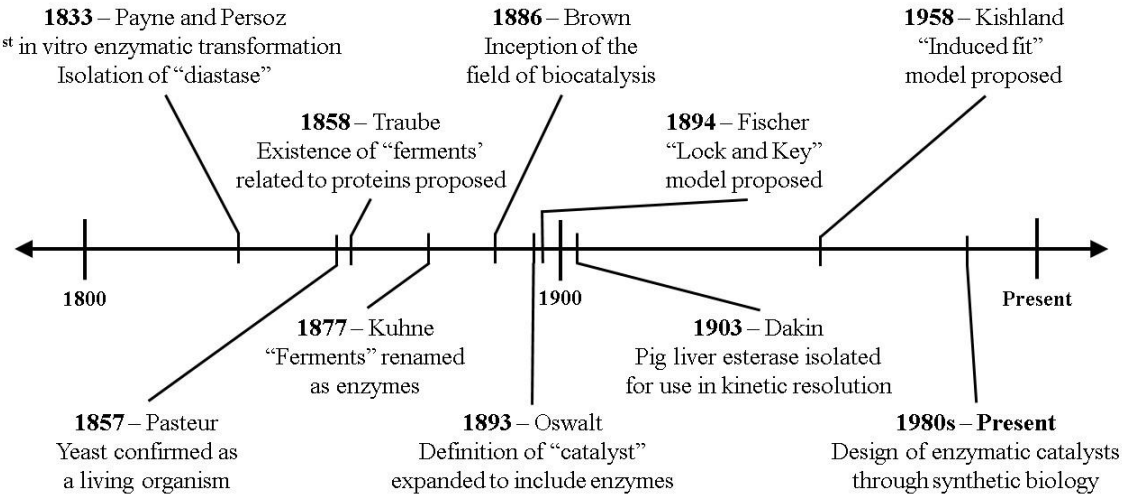


Figure 6: Timeline of the major accomplishments in the field of biocatalysis.

With more enzymes isolated and characterized, a classification system was developed in order to organize enzymes based upon the type of transformation they catalyze.[37] The nomenclature used for the classes of enzymes was derived from the terminology used by Payne and Persoz in 1833.[18] The six primary classes of enzymes are outlined in Table 1.[37]

Table 1: Classes of enzymes and the types of transformations they catalyze.[37]

Enzyme Class	**Examples of transformations catalyzed**
Oxidoreductase	Reduction of carbonyls/alkenes; Oxidation of C-H, C=C, C-N, C-O bonds; Cofactor oxidation/reduction
Transferase	Transfer of functional groups (i.e. acyl, glycosyl, aryl, methyl, phosphoryl, nitro, amino, etc.) from donor to acceptor
Hydrolase	Hydrolysis of esters, amides, lactones, anhydrides, nitriles, etc.; Formation of esters, amides, lactones, etc.
Lyase	Cleavage of C-C, C-O, C-N, C-S, C-X and P-O bonds without energy input from adenosine triphosphate (ATP)
Isomerase	Intramolecular rearrangements (isomerization, epimerization, racemization), intramolecular oxidation/reduction
Ligase	Bond formation (C-C, C-O, C-N, C-S, etc.) with energy input from ATP

In addition to the application of known enzymatic catalysts in novel ways, new enzymes are continuously being identified both from natural sources and from the design of these catalysts through synthetic biology.[36] The continuing advancement in the field of biocatalysis will ensure that enzymatic catalysts remain important in the field of organic synthesis

2.2 Arene Dioxygenases

The following section will provide a brief discussion of the role of oxidoreductase enzymes in the oxidation of arenes. The dioxygenase enzymes TDO and BZDO will discussed in detail.

2.2.1 Oxidoreductase Enzymes in Arene Oxidation

Oxidoreductase enzymes are a broad class of catalysts that facilitate a wide variety of reactions.[37] In the context of arene oxidation, oxygenases represent a particularly important subset of oxidoreductase enzymes. Oxygenases are enzymes that transfer oxygen atom(s) from molecular oxygen to the given substrate.[38] Oxygenases can be divided into two classes: monooxygenases, which transfer one atom of molecular oxygen to a substrate; and dioxygenases, which transfer both oxygen atoms of molecular oxygen.[38] Dioxygenase enzymes typically make use of a metal-ion cofactor in order to facilitate the activation of molecular oxygen and to establish an oxidation-reduction cycle.[38]

The metabolism of aromatic substrates by microorganisms was first reported by Stormer, who in 1908 isolated *Bacillus hexacarbovorum*, which metabolized xylene and toluene.[39,40] In 1914, Wagner identified multiple strains of *Bacterium benzoli* that metabolized xylene, toluene, benzene, naphthalene and phenol.[40,41] The intermediate(s) produced in the metabolism of aromatics by dioxygenases were not known until the isolation of 1,2-dihydronaphthalene-1,2-diol (**16**) from the urine of naphthalene (**15**)-fed rats by Young in 1947 (Figure 7).[42] Haccius isolated pyrocatechol (**18**) as the major product of the metabolism of benzene (**17**) by *Nocardia coralline* in 1957,[43] providing

further understanding of this process. The mammalian dehydrogenation of 3,5-cyclohexadiene-1,2-diol (**19**) to pyrocatechol (**18**)[44] allowed for the connection of the observations made by Young and Haccius. Based upon these observations, in 1961 Marr and Stone proposed *trans*-cyclohexa-3,5-diene-1,2-diol (**20**) as a likely intermediate in the bacterial oxidation of benzene (**17**) (Figure 7).[45] In accordance with the proposal of *trans*-cyclohexa-3,5-diene-1,2-diol (**20**) as a likely intermediate, an epoxide intermediate was proposed as a precursor leading to the production of catechols (Figure 7).[46,47]

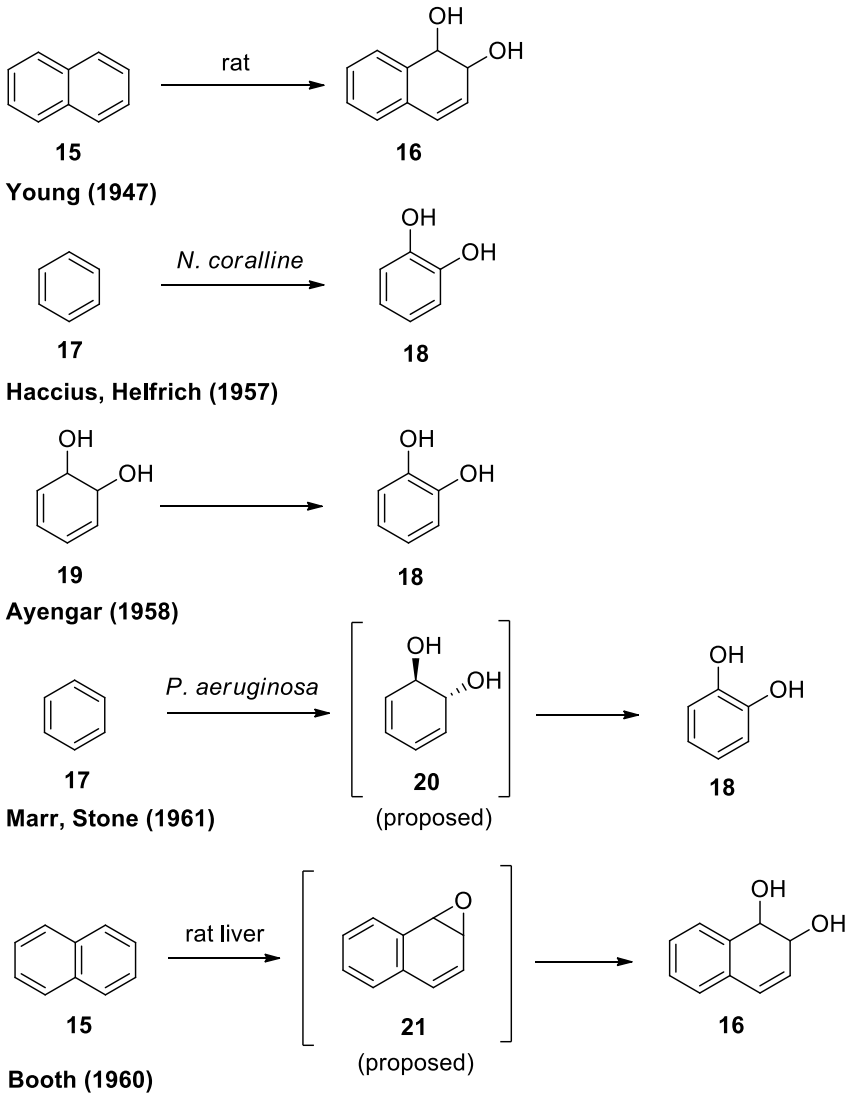


Figure 7: Early studies on the metabolism of aromatics by dioxygenase enzymes.[42-46]

In 1968, Gibson reported the oxidative metabolism of benzene by *Pseudomonas putida*.[48] In this report, Gibson suggests the *cis*-addition of molecular oxygen to the substrate, as was proposed by Kobayashi in the degradation of anthranilic acid (**22**) to pyrocatechol (**18**) (Figure 8).[49] Gibson also disproved the existence of the hypothesized epoxide and trans-dihydroxy intermediates by feeding *Pseudomonas putida* cultures with synthetic *trans*-3,5-cyclohexadiene-1,2-diol (**20**). These experiments demonstrated the inability of the bacteria to metabolize this compound, proving that this compound could not be an intermediate in the production of pyrocatechol (**18**) from benzene (**17**).[48] A second publication by Gibson in 1968 demonstrated the metabolism of halogenated aromatics by *P. putida* to the corresponding 3-halocatechols.[50] In the course of this study, Gibson isolated (+)-*cis*-4-chloro-2,3-dihydroxy-1-methylcyclohexa-4,6-diene (**25**) and 4-chloro-2,3-dihydroxy-1-methylbenzene (**26**) as the products of the metabolism of *p*-chlorotoluene (**24**) (Figure 8).[50] The isolation of (+)-*cis*-4-chloro-2,3-dihydroxy-1-methylcyclohexa-4,6-diene (**25**) confirmed the proposed *cis*-addition of molecular oxygen to the aromatic substrate. ^{18}O-labling studies demonstrated that both atoms of molecular oxygen are incorporated into the substrate.

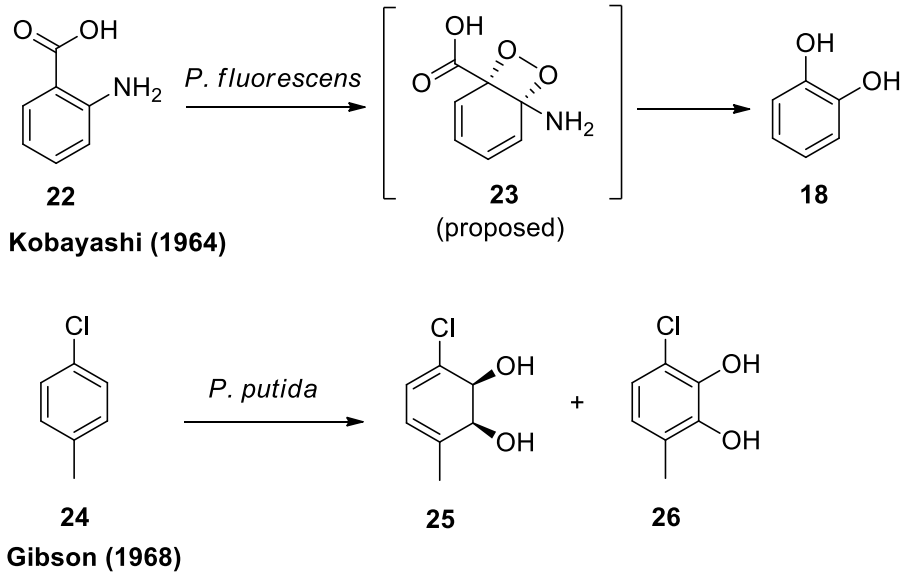
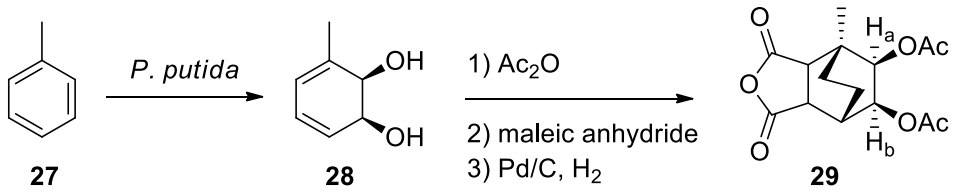


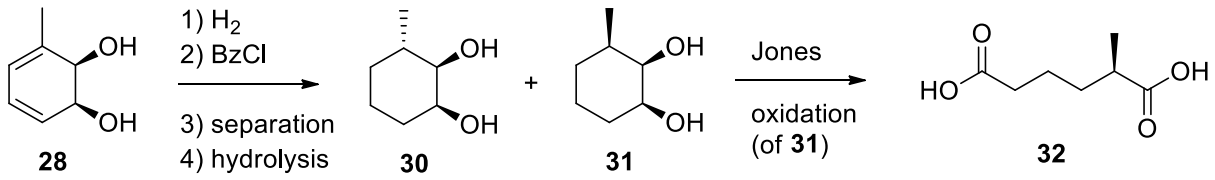
Figure 8: Studies on the metabolism of aromatics by dioxygenase enzymes.[49,50]

In order to conclusively prove the relative stereochemistry of the intermediates of type **25**, toluene (**27**) was metabolized by *P. putida* to (+)-*cis*-2,3-dihydroxy-1-methylcyclohexa-4,6-diene (**28**) (Scheme 1).[51] This compound is only available in small quantities from fermentation cultures of wild-type *P. putida* as it is readily metabolized to 3-methylcatechol. In order to produce metabolite **28** in larger quantities, a mutant strain (*P. putida* 39/D) was developed by Gibson to permit the accumulation of metabolite **28** in fermentation cultures.[51] The mutant strain was used to produce **28** which was then acetylated, treated with maleic anhydride and hydrogenated to produce cycloadduct **29** (Scheme 1).[51] The value of the coupling constant between H_a and H_b (8.5 Hz, Scheme 1) provided the final proof of the *cis* relationship between the hydroxyl groups in metabolites of type **28**.



Scheme 1: Derivatization of metabolite **28** for the determination of relative stereochemistry.[51]

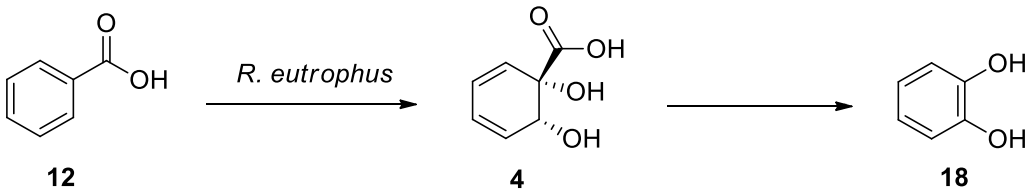
The absolute stereochemistry of **28** was confirmed through derivatization to a compound with known absolute stereochemistry, (*R*)-(−)-methyladipic acid (**32**) (Scheme 2).[52] This process involved the hydrogenation and mono-benzoylation of **28** to afford a mixture, which was separated and hydrolyzed to afford diastereomers **30** and **31**. The *cis,cis* relative stereochemistry of compound **31** was confirmed by NMR analysis, and this compound was subjected to oxidation to afford (*R*)-(−)-methyladipic acid (**32**). By matching the optical rotation of (*R*)-(−)-methyladipic acid (**32**) produced from the degradation of metabolite **28** to the reported value in the literature, Gibson provided the final proof of the absolute stereochemistry.[52]



Scheme 2: Derivatization of metabolite **28** for the determination of absolute stereochemistry.[52]

With the development of *P. putida* 39/D, it became possible to produce metabolites of type **28** in larger quantities.[51] In small scale biotransformations (0.5 L), the use of this organism allowed for the production of ~300 mg of diol metabolite per litre of fermentation culture.[53] In 1989, Gibson developed a recombinant *E. coli* strain (JM109 pDTG601A) expressing toluene dioxygenase.[54] The use of the recombinant strain in larger biotransformations (10-15 L) further increased the quantity of metabolites which can be isolated, with yields of >15 g/L for certain substrates.[14(m)] The availability of these compounds led to wide variety of chemical applications for metabolites of this type, as will be discussed in detail in subsequent sections.

In 1971, a similar study was performed by Reiner, working with *Alcaligenes eutropha* (later renamed *Ralstonia eutropha*).[55] *Alcaligenes eutropha* was shown to metabolize benzoic acid (**12**) to pyrocatechol (**18**) in an enzymatic process which proceeds through 1,2-dihydro-1,2-dihydroxybenzoic acid (**4**) (Scheme 3). In order to permit the accumulation of *ipso*-diol (**4**) in fermentation cultures, a mutant strain was employed (*Ralstonia eutropha* B9), in which the conversion of this compound to pyrocatechol (**18**) was blocked.[55] This mutant strain was developed and provided by Johnson.[55] ^{18}O-labeling studies demonstrated that both atoms of molecular oxygen were incorporated into the substrate,[55] as demonstrated by Gibson in the metabolism of aromatics by *P. putida*.[51] Based upon qualitative experiments, Reiner proposed a *cis* relationship between the hydroxyl groups of *ipso*-diol (**4**), in agreement with Gibson's results.[51]



Scheme 3: Metabolism of benzoic acid by *R. eutropha* (previously *A. eutropha*).[55]

Early observations indicated a *cis* relationship between the hydroxyl groups of *ipso*-diol (**4**),[55] and the absolute stereochemistry of this compound was conclusively proven in 1995.[56] Widdowson proved the absolute stereochemistry of *ipso*-diol (**4**) (Scheme 3) by conversion to its corresponding (*p*-bromobenzoyl)methyl ester and x-ray diffraction analysis of this compound.[56]

Despite the seminal work by Gibson, Reiner and many others, the identity of the dioxygenase enzymes involved in these processes were not known when the *Pseudomonas putida* 39/D and *Ralstonia eutropha* B9 strains were developed. It was later determined that the enzyme responsible for catalyzing the 2,3-dihydroxylation observed in *P. putida* 39/D is toluene dioxygenase (TDO)[12] and benzoate dioxygenase (BZDO) catalyzes the 1,2-dihydroxylation observed in *R. eutropha* B9.[13]

The nature of these enzymes, the mechanism by which they catalyze the dihydroxylation of aromatics, their structure, substrate scope and selectivity will be discussed in the following sections.

2.2.2 Toluene Dioxygenase (TDO)

The following section will provide a discussion on the structure, mechanism, substrate scope, selectivity and applications of toluene dioxygenase (TDO).

2.2.2.1 Structure and Mechanism of Catalysis

Toluene dioxygenase (TDO) catalyzes the 2,3-dihydroxylation of aromatic compounds (Figure 9).[12] The selectivity depicted in Figure 9 will be discussed in detail in Section 2.2.2.2.[57] The extensive research performed by Gibson and others has elucidated the nature and absolute stereochemistry of the metabolites produced by this enzyme. However, the mechanism by which the dihydroxylation occurs remains a subject of debate. In order to discuss the mechanism of this transformation, it is necessary to understand the structure of the toluene dioxygenase system and its key catalytic subunits.

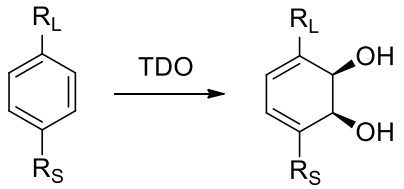


Figure 9: Metabolism of substituted aromatics by TDO.[57,12]

TDO belongs to a class of dioxygenase enzymes referred to as Rieske non-heme iron dioxygenases.[58] This classification indicates that TDO contains a Rieske iron-sulfur cluster [2Fe-2S] as well as a mononuclear iron atom in the catalytic subunit.[59] Although "toluene dioxygenase" is often used colloquially to refer to this enzyme, the TDO system in fact consists of three enzymes: the terminal dioxygenase (TDO-O), a Rieske ferredoxin

(TDO-F) and a reductase (TDO-R). The terminal dioxygenase (TDO-O) is a hexamer of three β-subunits and three α-subunits, with each α-subunit containing a catalytic site (Figure 10).[59]

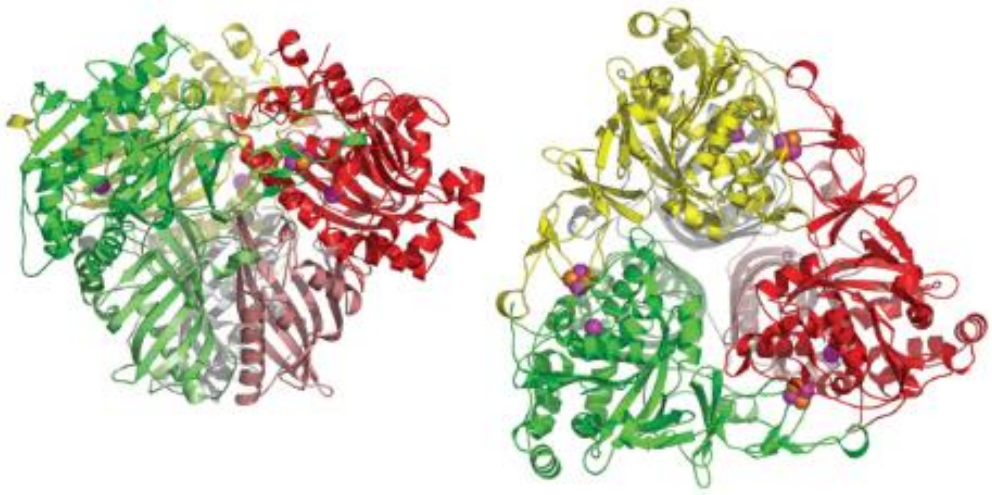


Figure 10: Structure of TDO-O hexamer (α-subunits = red, green, yellow; β-subunits = pink, light green, gray; Fe = magenta, S = orange).[59]

Electrons are transferred from nicotinamide adenine dinucleotide (NADH) to flavin adenine dinucleotide (FAD) in TDO-R where they are then passed on to TDO-F, which shuttles the electrons to the terminal dioxygenase, TDO-O.[59] Within TDO-O, a Rieske iron-sulfur cluster receives the electrons from TDO-F and then passes them on to a mononuclear iron atom through a highly conserved aspartate residue (Figure 11).[58,59] It is clear that the role of the loosely-bound mononuclear iron atom in TDO-O, coordinated by two histidine residues and one aspartate, is important in the catalytic mechanism, as the loss of this atom results in a complete loss of activity.[59] This follows from the fact that the mononuclear iron is known to be the site of oxygen and substrate binding,[58,59] but

the exact role of the iron atom in the mechanism of the dihydroxylation however is still debated.

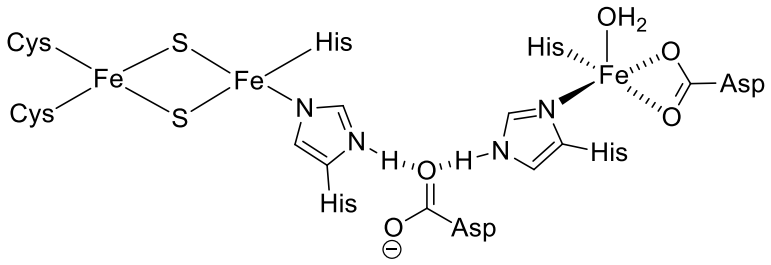


Figure 11: Active site configuration of TDO.[58,59]

Multiple proposals have been made as to the mechanism of the enzymatic dihydroxylation. These hypotheses include the formation of a dioxetane intermediate (**35**) with singlet oxygen ($^1O_2$),[60] as well as the formation of intermediates **35** and **36** through a [3+2] cycloaddition with an iron-peroxide species (Figure 12).[14(r)] A covalently bound iron-peroxide intermediate of type **38** has also been proposed,[61] as has the formation of iron-peroxide **39** leading to iron-peroxo species **40** (Figure 12).[62]

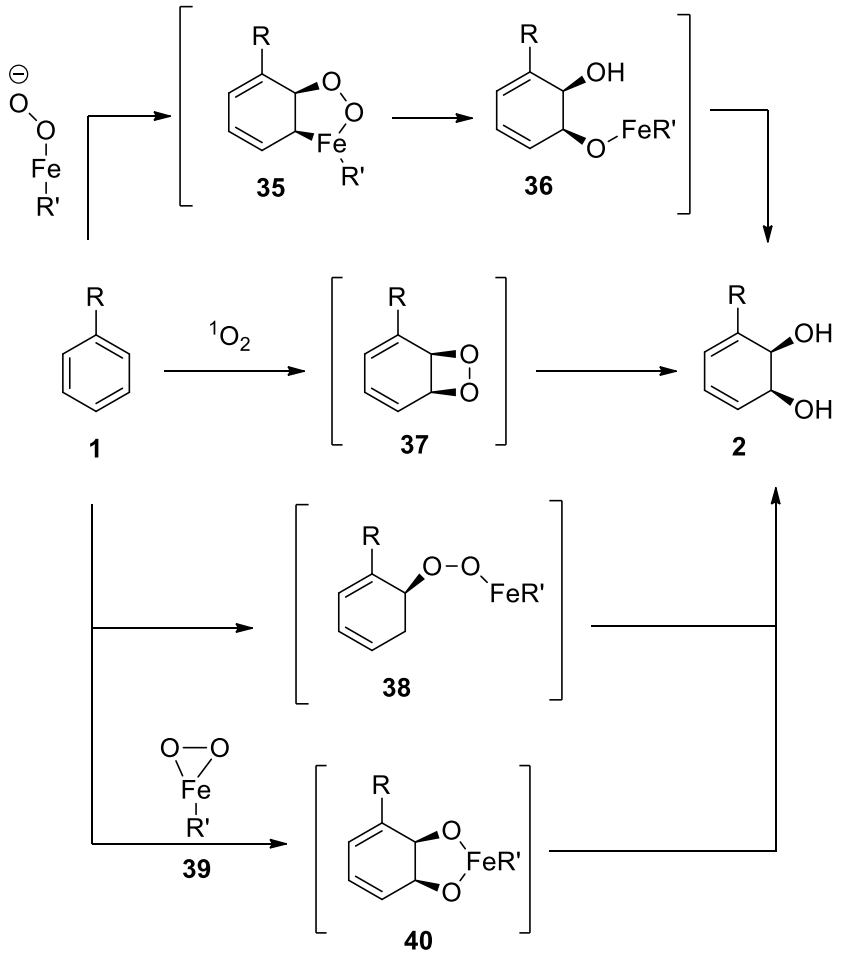


Figure 12: Mechanistic proposals for dioxygenase-mediated dihydroxylation.[14(r),60-62]

The mechanism involving the formation of dioxetane **37**, first suggested by Gibson in 1970, would involve a 2 electron reduction of the dioxetane by the enzyme.[60] This mechanism is unlikely however, as the presence of iron-sulfur clusters indicates single electron transfer in the mechanism.[38] Furthermore, this mechanism would require the activation of triplet oxygen to high-energy singlet oxygen.[38]

The proposal involving the formation of intermediates **35** and **36**, though consistent with single electron transfer in the catalytic mechanism, has yet to be supported by any experimental evidence.[14(p)]

The existence of the covalently bound iron-peroxide of type **38** was first proposed by Ramaswamy in 2000, based upon the study of naphthalene dioxygenase (NDO).[61] This mechanistic proposal is supported by the observation that both NDO and TDO can perform the monohydroxylation of certain substrates.[63] It is also supported by the fact that oxygen activation in NDO can be decoupled from substrate hydroxylation, suggesting a stepwise rather than a concerted mechanism.[64]

Upon further study of the catalytic mechanism of NDO, Ramaswamy proposed a mechanism involving the iron-peroxo species **40** in 2003.[62] This mechanism was based on the observation of "side-on" binding of oxygen to iron in the crystal structure of NDO, leading to the formation of peroxide species **39**.[62] Based upon the crystal structures of NDO with substrate bound, with oxygen bound, with substrate and oxygen bound, and with product bound, the catalytic mechanism shown in Figure 13 was proposed.

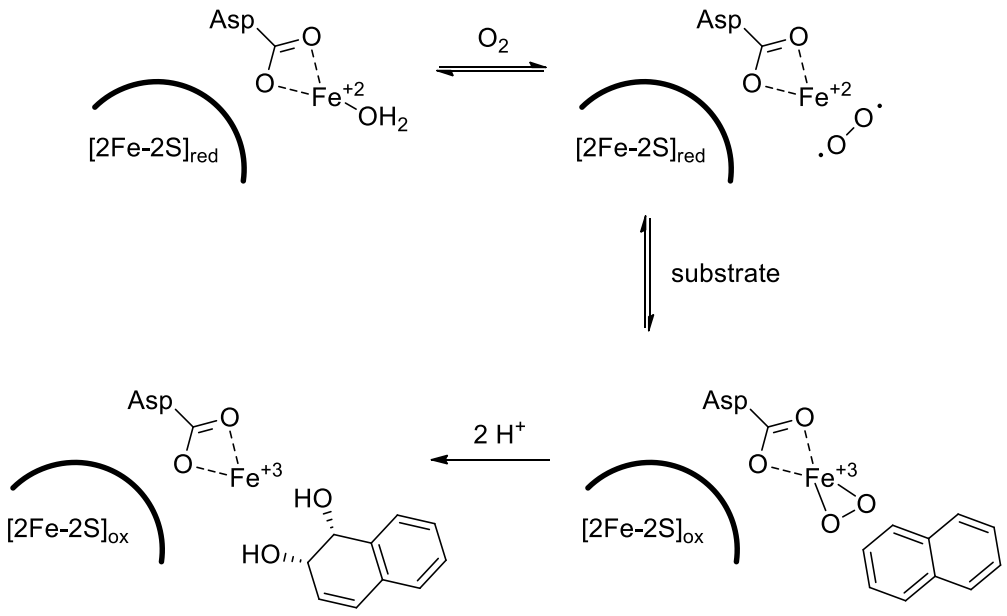


Figure 13: Mechanistic proposal for NDO-mediated dihydroxylation.[62]

There is no current consensus regarding the mechanism of dioxygenase enzymes such as TDO and NDO.[14(r)]

2.2.2.2 Substrate scope and selectivity

With the development of the mutant strain *P. putida* 39/D,[51] the investigation of the substrate scope of TDO and the isolation of any new metabolites produced was greatly expedited. Isolation of metabolites was complicated, however, by the need to induce the up-regulation of TDO with an aromatic inducer.[51] This would invariably result in a mixture of metabolites upon dihydroxylation of the inducer and the substrate of interest. To resolve this issue, a recombinant *E. coli* strain (JM109 pDTG601A) was developed that did not require the use of an aromatic inducer.[54]

P. putida 39/D and *E. coli* (JM109 pDTG601A) have been used to study the substrate scope of TDO,[14(o)] and this topic has been comprehensively reviewed up to 2004.[14(o)] A small representative sample of the metabolites that have been isolated is shown in Figure 14. TDO has been shown to accept a wide variety of monosubstituted aromatics, producing metabolites of type **41**-**45**. TDO also accepts *ortho*-, *meta*- and *para*-disubstituted aromatics (**46**-**49**) as well as trisubstituted aromatics (**50**).[14(o)] Biphenyls (**51**) and some heteroaromatics (**52**) have also been shown to be substrates for TDO.[14(o)]

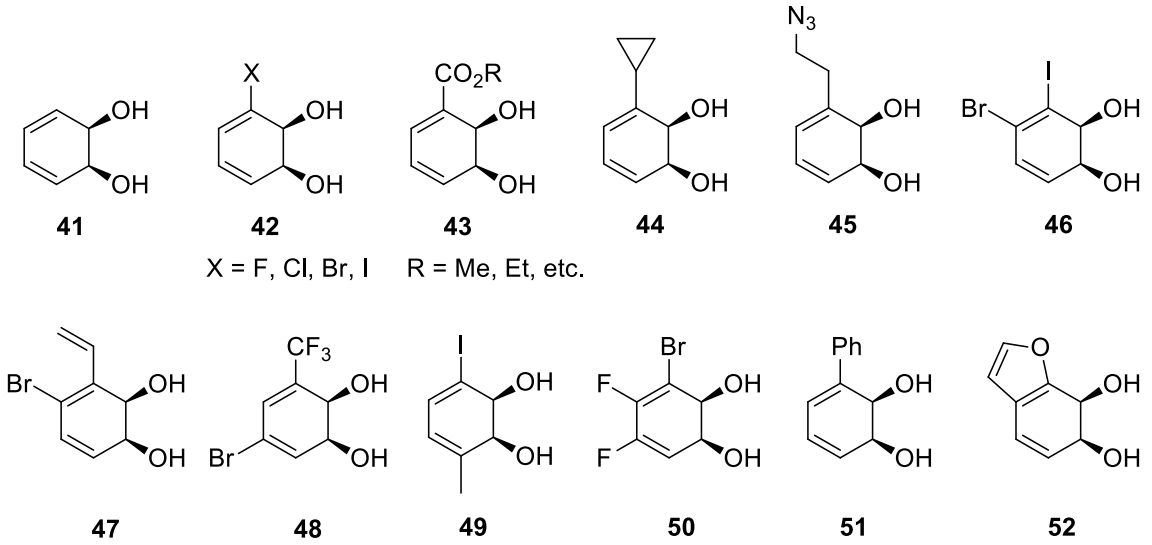


Figure 14: Examples of metabolites produced by TDO.[14(o)]

Despite the relative promiscuity of TDO, the substrate scope has been shown to be limited by the size and polarity of the substrate.[65] Bulky substrates such as *t*-butyl benzoate (**53**)[66] and polar substrates such as the heterocyclic picoline **55**[67] have been shown not to be substrates for TDO (Figure 14).

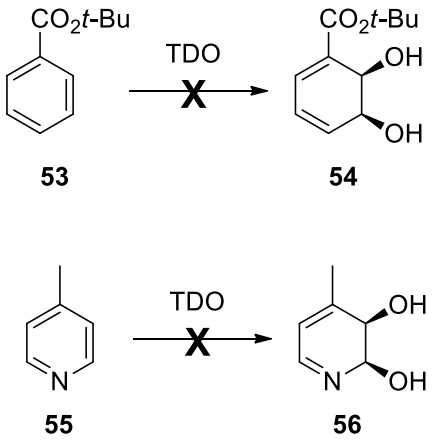


Figure 15: Limitations in the substrate scope of TDO.[66,67]

The large substrate scope observed in the study of TDO has led to the continued investigation of the limits of the substrate scope and the isolation of unique metabolites. In the TDO-mediated dihydroxylation of arenes with multiple substituents (**57**, **60**, **63**), two isomeric metabolites can be produced, as shown in Figure 16.[57,68]

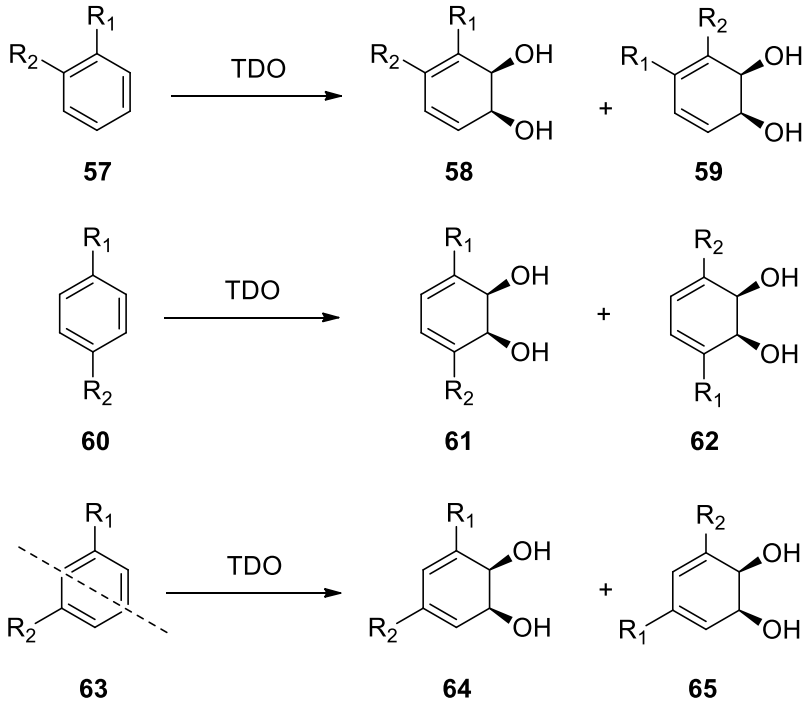
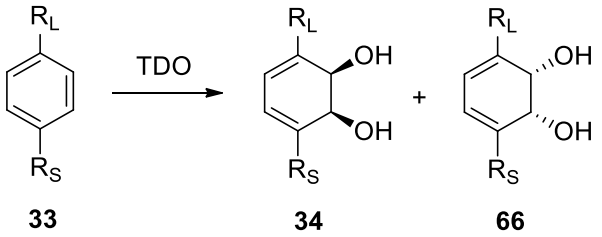


Figure 16: Metabolites produced in dihydroxylation of multiply-substituted arenes.[57,68]

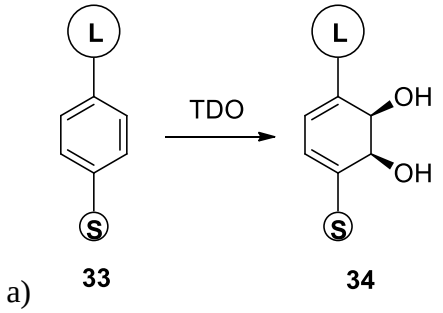
In order to develop a predictive model for the outcome of these biotransformations, Boyd investigated a series of *para*-disubstitued arenes (**33**) as substrates for TDO (Figure 17).[57] In this study, the steric size of the arene substituents was varied and the enantiomeric excess of the metabolites produced (**34** : **66**) was analyzed (Figure 17).[57]



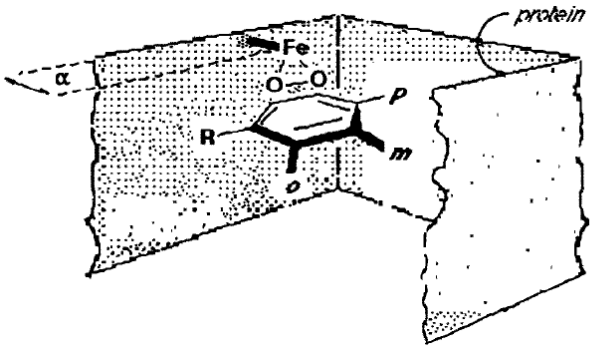
R_L	R_S	34 : 66
CF_3	Me	99 : 1
CF_3	I	99 : 1
I	F	94 : 6
Me	F	92 : 8
I	Me	90 : 10
I	F	80 : 20

Figure 17: Dihydroxylation of *para*-disubstituted arenes and ratio of metabolites.[57]

From these data, Boyd determined that the sterically larger substituent dictated the facial selectivity of the enzymatic dihydroxylation.[57] On the basis of this study, a model was proposed which could be used to predict the outcome of the dihydroxylation of multiply-substituted arenes (Figure 18a).[57] This model was later visualized in the context of the TDO active site by Hudlicky (Figure 18b).[68(b)]



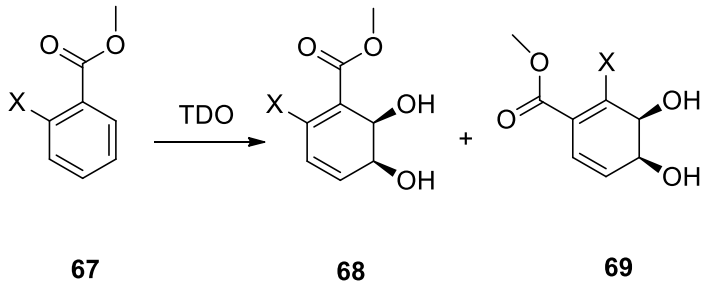
a)



b)

Figure 18: Model for the dihydroxylation of multiply-substituted arenes.[57,68(b)]

This model was later examined in the context of *ortho*-disubstituted arenes by Hudlicky.[69] By studying the dihydroxylation of *ortho*-halo methyl benzoates (**67**), it was shown that sterically larger substituents have a stronger directing effect on the dihydroxylation (Figure 18).[69] In this way, Hudlicky confirmed the applicability of Boyd's model[57] to *ortho*-disubstituted arenes (Figure 19).



X	68 : 69
F	100 : -
Cl	4 : 1
Br	3.5 : 1
I	1 : 4

Figure 19: Dihydroxylation of *ortho*-disubstituted arenes and ratio of metabolites.[69]

The validity of Boyd's model[57] was further supported in 2009 when TDO was co-crystallized with toluene.[59] The resulting crystal structure (Figure 20) demonstrated the alignment of the positions *ortho*- and *meta*- to the sterically largest substituent with modelled dioxygen.[59] This visualization of the active site with bound substrate served to explain the selectivity of the dihydroxylation and the *cis* relationship of the hydroxyl groups in the metabolites produced.[59]

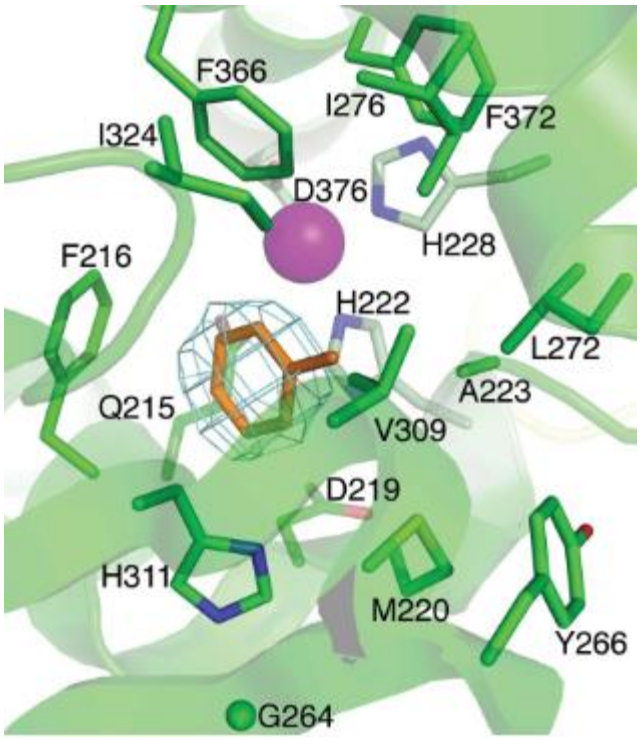
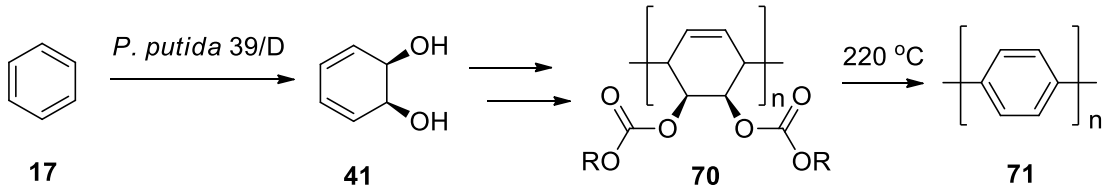


Figure 20: Crystal structure of TDO active site with bound substrate (toluene) (Red ball = mononuclear Fe center).[59]

2.2.2.3 Use of *cis*-cyclohexadienediols in organic synthesis

As discussed in the Introduction, biocatalysis has the potential to significantly increase the efficiency of a synthetic sequence. In the synthetic application of *cis*-cyclohexadienediols, this potential was first recognized by Imperial Chemical Industries (ICI, now AzkoNobel) in 1983.[70] ICI applied the *meso*-diol (**41**) derived from the dihydroxylation of benzene by *P. putida* in the synthesis of polyphenylene (**71**) (Scheme 4).[70]

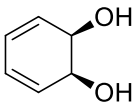
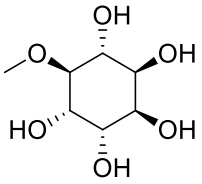
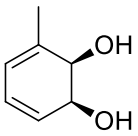
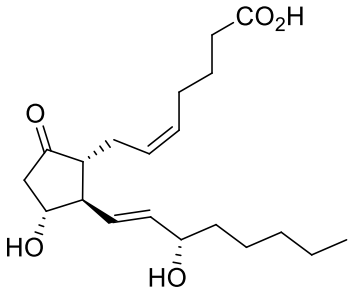
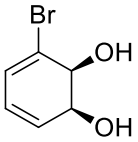
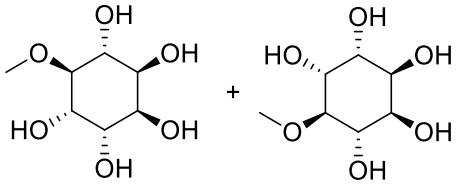
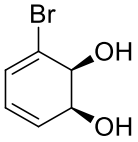
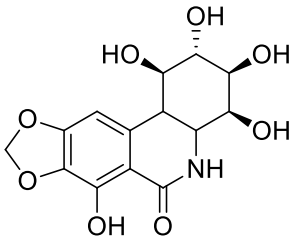
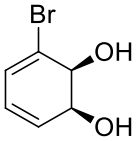
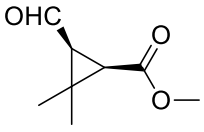


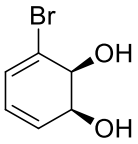
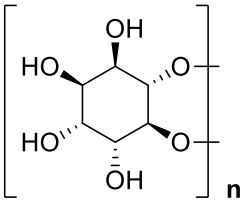
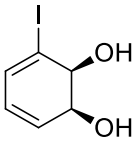
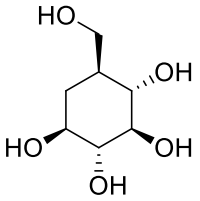
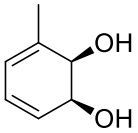
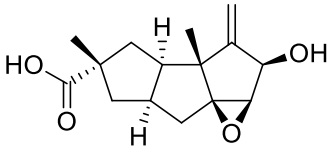
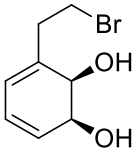
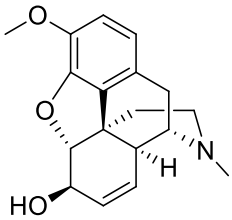
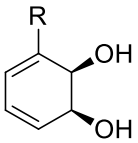
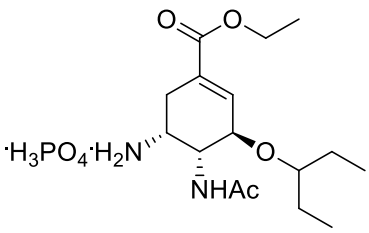
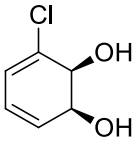
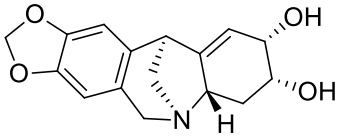
Scheme 4: Synthesis of polyphenylene (**66**) by Imperial Chemical Industries.[70]

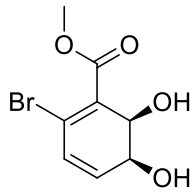
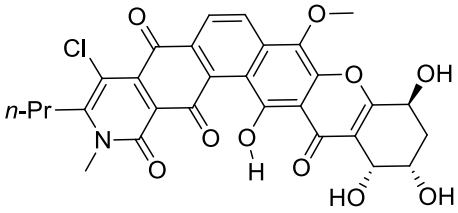
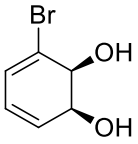
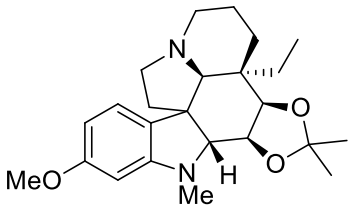
The synthetic application of *cis*-cyclohexadienediol metabolites was first adopted in academia by Ley in 1987.[71] Ley also used the *meso*-diol **41** to synthesize (±)-pinitol (**72**) in just six steps.[71] Shortly after this seminal publication, Hudlicky applied *cis*-cyclohexadienediols in synthesis for the first time in North America.[72] Hudlicky utilized the metabolite of toluene (**28**) in a short formal synthesis of prostaglandin ($PGE_{2\alpha}$, **73**).[72] In 1990, Hudlicky demonstrated the versatility of *cis*-cyclohexadienediols in enantioselective synthesis by applying the metabolite of bromobenzene (**74**) in the synthesis of both (+)- and (−)-pinitol (**72**).[73]

These early reports led many synthetic chemists to recognize the potential utility of *cis*-cyclohexadienediol metabolites.[14] A small, representative sample of the syntheses which have originated in *cis*-cyclohexadienediol metabolites is given in Table 2.[71-85]

Table 2: Examples of chemoenzymatic syntheses using *cis*-cyclohexadienediols.[71-85]

Starting material	Product	Author	Year	Reference
 **41**	 (±)-pinitol (**72**)	Ley	1987	[71]
 **28**	 Prostaglandin ($PGE_{2\alpha}$, **73**) (Formal Synthesis)	Hudlicky	1988	[72]
 **74**	 (+)- and (−)-pinitol (**72**)	Hudlicky	1990	[73]
 **74**	 (+)-pancratistatin (**75**)	Hudlicky	1995	[74]
 **74**	 **76**	Banwell	1996	[75]

 **74**	 oligoinositols (**77**)	Hudlicky	2002	[76]
 **78**	 carba-β-L-glucopyranose (**79**)	Boyd	2005	[77]
 **28**	 (+)-hirsutic acid (**80**)	Banwell	2007	[78]
 **81**	 (+)-codeine (**82**)	Hudlicky	2007	[79]
 R = Br (**74**)[80,81] R = CO_2Et (**83**)[82]	 Tamiflu (**84**)	Fang, Banwell, Hudlicky	2008 2008 2009	[80] [81] [82]
 **85**	 (+)-nangustine (**86**)	Banwell	2008	[83]

 **87**	 Kibdelone A (**88**)	Porco, Hudlicky	2013	[84]
 **74**	 Vindoline analogue (**89**)	Banwell	2016	[85]

Despite the fact that more than 400 *cis*-cyclohexadienediol metabolites have been identified,[14(o)] only a small fraction (< 10) of these have been applied in synthesis.[14(r)] The fact that so many metabolites have yet to be applied, combined with the versatility of these compounds, leaves tremendous potential for continued advancement in this field.

2.2.3 Benzoate dioxygenase (BZDO)

The following section will provide a discussion on the structure, mechanism, substrate scope, selectivity and applications of benzoate dioxygenase (BZDO).

2.2.3.1 Structure and Catalytic Mechanism

Benzoate dioxygenase (BZDO) catalyzes the 1,2-dihydroxylation of benzoic acid and related derivatives (Figure 21).[13] Like TDO, BZDO is a Rieske non-heme iron dioxygenase system which is made up of multiple protein components.[86] The BZDO system consists of a reductase enzyme (BZDO-R) and a terminal oxygenase (BZDO-O).[86] Unlike TDO, the BZDO system lacks a distinct ferredoxin protein.[86]

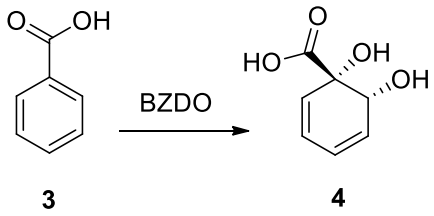


Figure 21: Metabolism of benzoic acid (**3**) by BZDO.[13]

Despite lacking a distinct ferredoxin protein, electron transport in BZDO occurs in a very similar manner as in TDO (discussed in Section 2.2.2.1).[58,87] BZDO-R consists of three domains; an NADH binding domain, a flavin binding domain, and a ferredoxin domain (Figure 22).[87] As in TDO, electrons are transferred from NADH to FAD within the reductase, where they are then passed on to the Rieske [2Fe-2S] centre in the ferredoxin domain, and finally on to the terminal oxygenase.[87]

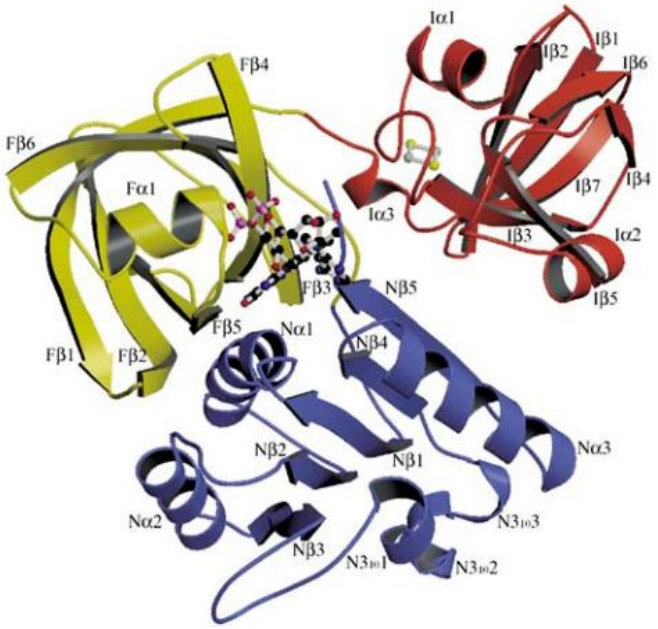


Figure 22: Structure of BZDO-R (FAD domain = yellow, NADH domain = blue, ferredoxin domain = red).[87]

Although no crystal structure is available for BZDO-O, this terminal oxygenase has been shown to be a hexamer of three β-subunits and three catalytic α-subunits.[86(c)] The structure of the catalytic site in the α-subunit can be drawn from analogy to other dioxygenase enzymes (Figure 11, Section 2.2.2.1), as this site has been shown to be highly conserved throughout this family.[58,59]

As in the case of TDO, the catalytic mechanism of BZDO remains the subject of debate. Careful study of the mononuclear iron centre in the catalytic site through EPR spectroscopy provided evidence of a reactive intermediate of type **39** (Figure 12, Section 2.2.2.1).[62] This finding, as well as evidence of the side-on binding of oxygen to the mononuclear iron centre observed in other dioxygenase enzymes,[59,62] have led to a proposed mechanism which is analogous to that shown in Figure 13 (Section 2.2.2.1).

2.2.3.2 Substrate scope and selectivity

Although the substrate scope of BZDO has been shown to be more limited than TDO, this enzyme system does accept a range of substituted benzoic acid derivatives.[14(o),15] Metabolism of *ortho*-, *meta*- and *para*-substituted benzoic acid derivatives by BZDO has led to the isolation of metabolites of type **90-96** (Figure 23).[14(o),15,88] BZDO also accepts a limited range of tri-substituted benzoic acid derivatives to produce metabolites of type **97-99** (Figure 23).[14(o),15]

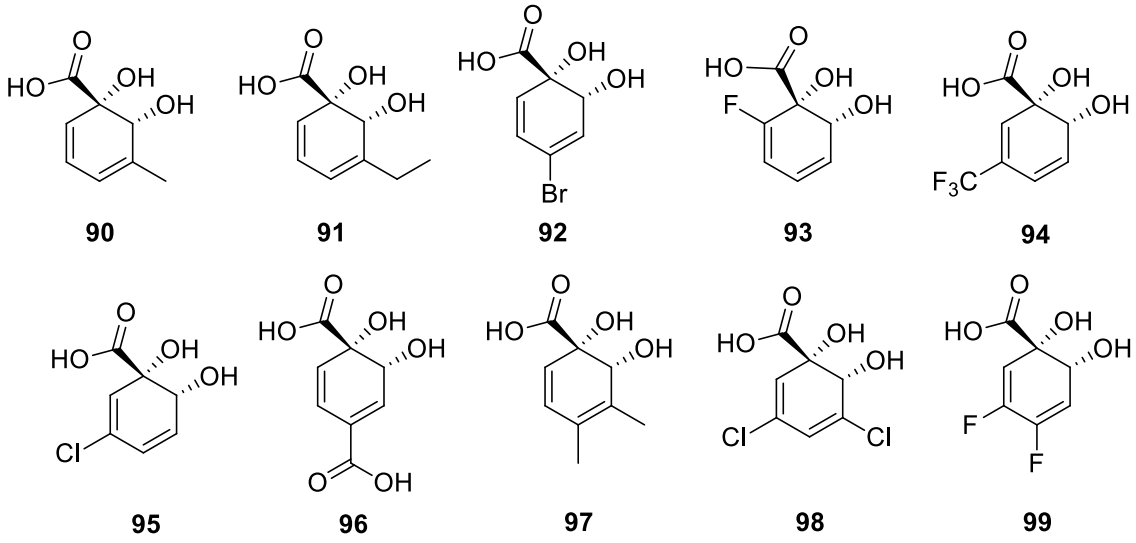
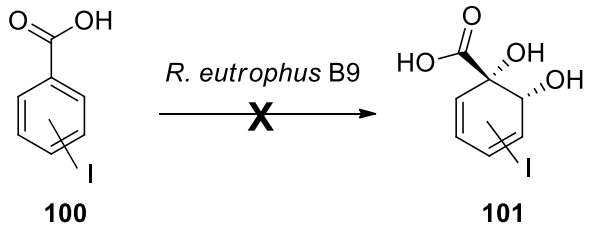


Figure 23: Examples of metabolites produced by BZDO.[14(o),15,88]

In his study of the metabolism of substituted benzoic acid derivatives as substrates for BZDO, Knackmuss demonstrated that substitution at the *meta* position is most well tolerated.[89] *Para* substitution was less well tolerated, with 4-methyl and 4-chlorobenzoic acid being metabolized only very slowly.[89] Substitution at the *ortho* position was the least well tolerated, with only 2-fluorobenzoic acid being metabolized.[89] The substrate

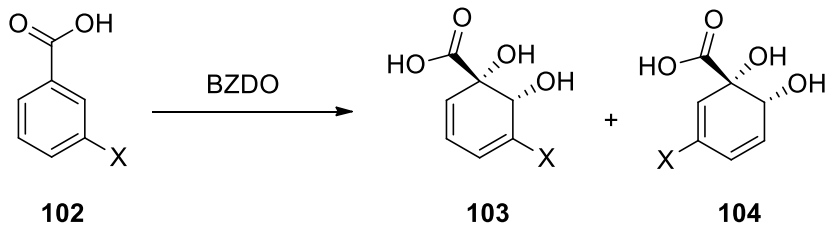
scope of BZDO has also been shown to be limited by the steric size of the substituents, as 2-,3- and 4-iodobenzoic acid (**100**) were shown not to be metabolized (Figure 24).[90]



100 **101**

Figure 24: Attempted dihydroxylation of iodobenzoic acids with BZDO.[90]

Boyd's model for the regioselectivity of dioxygenase-mediated dihydroxylation (Figure 18)[57] has been shown not to apply to BZDO, as the dihydroxylation is directed by the carboxylate moiety in all cases.[14(o),15] In the metabolism of *meta*-substituted benzoic acid derivatives, it was initially reported that 5-halo metabolites (**104**) were formed in preference to 3-halo metabolites (**103**) (Figure 25).[89(a)] This finding was revised in later studies, as it was shown that 3-halo metabolites (**103**) were preferentially produced in the metabolism of 3-methyl and 3-halobenzoic acids (Figure 25).[89(c),91]



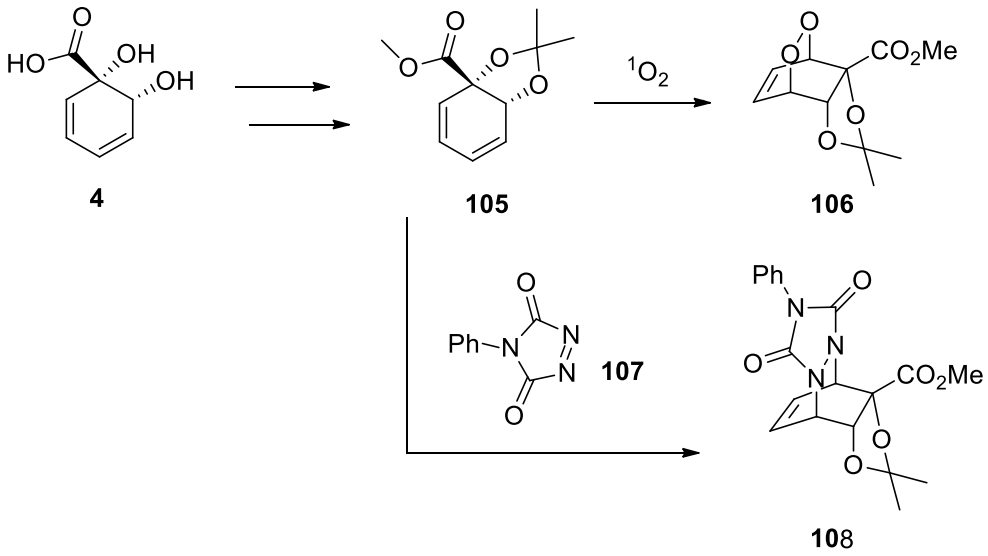
102 **103** **104**

X	103 : 104
F	1.4 : 1
Cl	2 : 1
Br	3 : 1
Me	9 : 1

Figure 25: Regioselectivity in the metabolism of *meta*-substituted benzoic acid derivatives.[89,91]

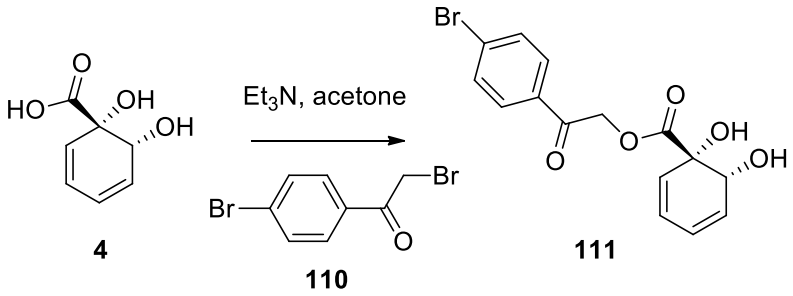
2.2.3.3 Use of *ipso*-cyclohexadienediols in organic synthesis

The first synthetic application of the metabolite of benzoic acid produced by BZDO was reported by Widdowson and Ribbons in 1995,[92] 24 years after this compound was first characterized.[55] This report demonstrated the stereo- and regioselectivity by a series of cycloaddition reactions between derivatives of metabolite **4** and various cycloaddition partners (Scheme 5).[92]



Scheme 5: First synthetic application of metabolite **4** by Widdowson and Ribbons.[92]

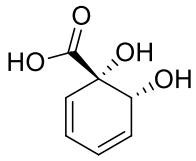
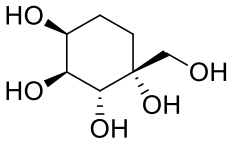
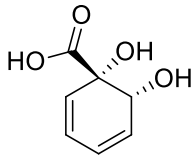
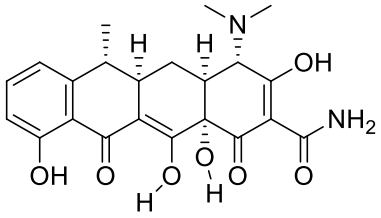
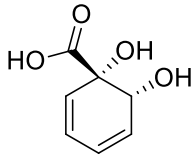
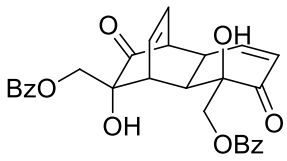
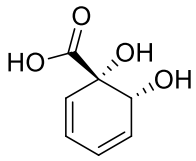
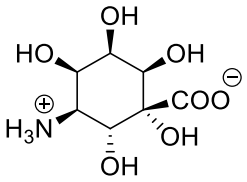
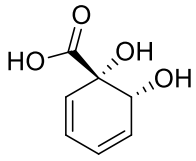
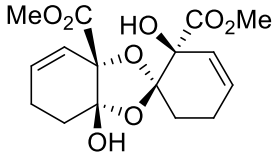
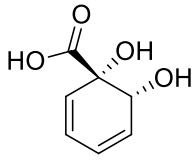
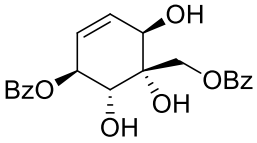
In the same report, the absolute stereochemistry of metabolite **4** was demonstrated through the formation of its (*p*-bromobenzoyl)methyl ester (**111**) (Scheme 6).[92] X-ray crystallographic analysis of ester **111** confirmed the absolute stereochemistry of metabolite **4** to be as shown in Scheme 6.[92]

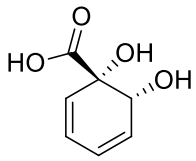
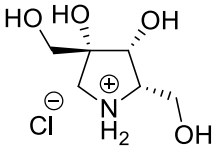


Scheme 6: Derivatization of metabolite **4** for the determination of absolute stereochemistry.[92]

The reactivity of metabolite **4** was further studied by Myers[93] and by Mihovilovic,[94] who demonstrated the versatility of this metabolite and described its potential synthetic utility. In 2004, the first total synthesis using metabolite **4** was reported by Parker at Johnson and Johnson.[95] This synthesis utilized *ipso*-diol **4** in the production of analogues of an anti-convulsant agent, an example of which is shown in Table 3.[95] The first natural product synthesis to use *ipso*-diol **4** was reported by Myers in 2005, in the synthesis of tetracycline antibiotics.[96] This synthesis represented a significant improvement in yield over previous approaches to tetracyclines,[15(a),96] and likely played a significant role in the recognition of the utility of *ipso*-diol **4**. Following this 2005 report, *ipso*-diol **4** has been applied in the synthesis of many compounds of interest,[15] relevant examples of which are shown in Table 3.[95-101]

Table 3: Examples of chemoenzymatic syntheses using *ipso*-diol **4**.[95-101]

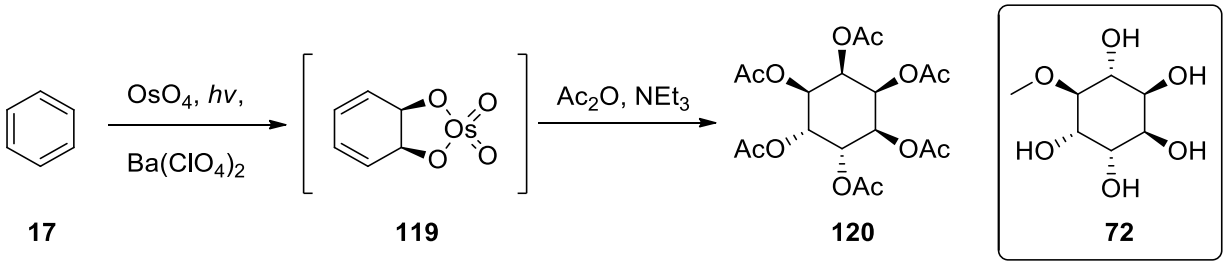
Starting Material	Product	Author	Year	Reference
 **4**	 topiramate analogue (**112**)	Parker	2004	[95]
 **4**	 (−)-6-deoxytetracycline (**113**)	Myers	2005	[96]
 **4**	 (+)-grandifloracin (**114**)	Lewis	2011	[97]
 **4**	 "inosaminoacid" (**115**)	Lewis	2011	[98]
 **4**	 (−)-idesolide (**116**)	Hudlicky	2011	[99]
 **4**	 (+)-zeylenol (**117**)	Lewis	2012	[100]

 **4**	 "iminosugar" (**118**)	Hudlicky	2014	[101]

As evident from Table 3, all of the reported total synthesis employing BZDO have used *ipso*-diol **4**. Reports by Banwell[88] and Lewis[90] demonstrated the versatility and potential utility of the metabolites of substituted benzoic acid derivatives (such as those shown in Figure 23). Despite these reports, none of these compounds have yet been applied in total synthesis. The fact that so many metabolites have yet to be applied, combined with the versatility of these compounds, leaves tremendous potential for continued advancement in this field.

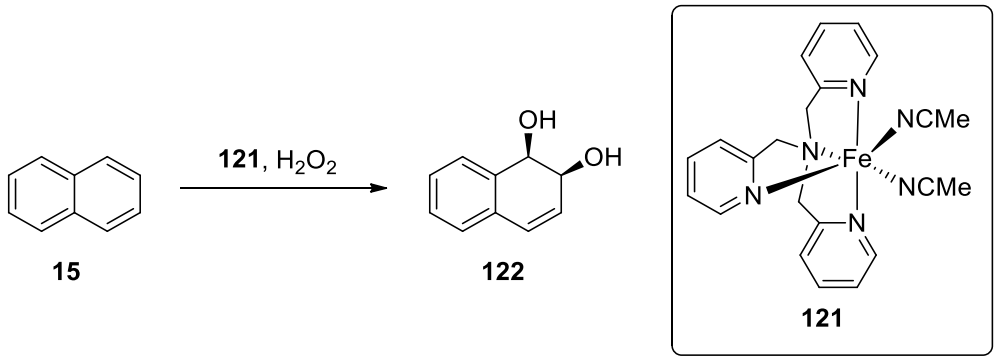
2.2.4 Synthetic Dioxygenase Analogues

As the dioxygenase-mediated *cis*-dihydroxylation of aromatics is one of the few natural transformations without a synthetic equivalent, efforts have been made to identify catalysts that mimic the functions of dioxygenases.[102,103] In 1995, Motherwell used osmium tetraoxide under light irradiation to achieve the catalytic dihydroxylation of benzene (**17**) (Scheme 7).[102(a)] The transformation was proposed to proceed through intermediate **119**, and was used in the synthesis of cyclitol derivatives such as **120**, and later in the synthesis of (±)-pinitol (**72**).[102]



Scheme 7: Catalytic dihydroxylation of benzene (**17**) by Motherwell.[102]

More recently, Que reported an iron-based catalyst which performed the *cis*-dihydroxylation of naphthalene with hydrogen peroxide in low yield (Scheme 8).[103] This catalyst system was used to study the mechanism of oxygen incorporation from hydrogen peroxide to diol **122**.[103]



Scheme 8: Catalytic dihydroxylation of naphthalene (**15**) by Que.[103]

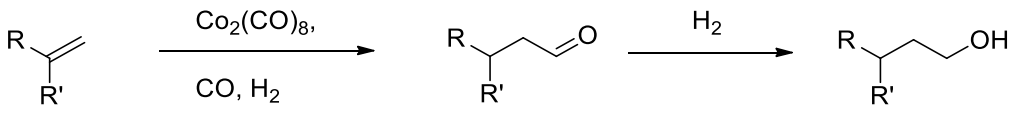
Although research in this field has identified multiple potential catalyst systems,[102,103] the enantioselective, or even racemic, dihydroxylation of aromatics with a synthetic dioxygenase mimic has yet to be achieved. Research in this field remains active.

2.3 Carbonylation Chemistry

One of the topics discussed in this thesis will be the chemical generation of metabolites that are produced in low yield by fermentation. In section 3.2, the synthesis of diols derived from benzoates in relatively low yield (< 1 g/L) will be presented. The diols derived from halobenzenes are produced in high yields (10-20 g/L). Conversion of these diols to those derived from benzoates is accomplished by palladium-catalyzed carbonylation. The following section provides a brief review of this methodology.

2.3.1 History

Carbonylation chemistry is a field with a long history dating to the discovery of the cobalt-catalyzed hydroformylation of olefins by Roelen in 1938 (Scheme 9).[104] Its importance is exemplified by the fact that the hydroformylation process was used in the industrial production of 10.4 million metric tons of compounds of type **124** and **125** as recently as 2008.[105] Because carbonylation chemistry is a tremendously varied and well-studied field, the discussion here will focus on the palladium-catalyzed carbonylation of aryl and vinyl halides and pseudohalides.

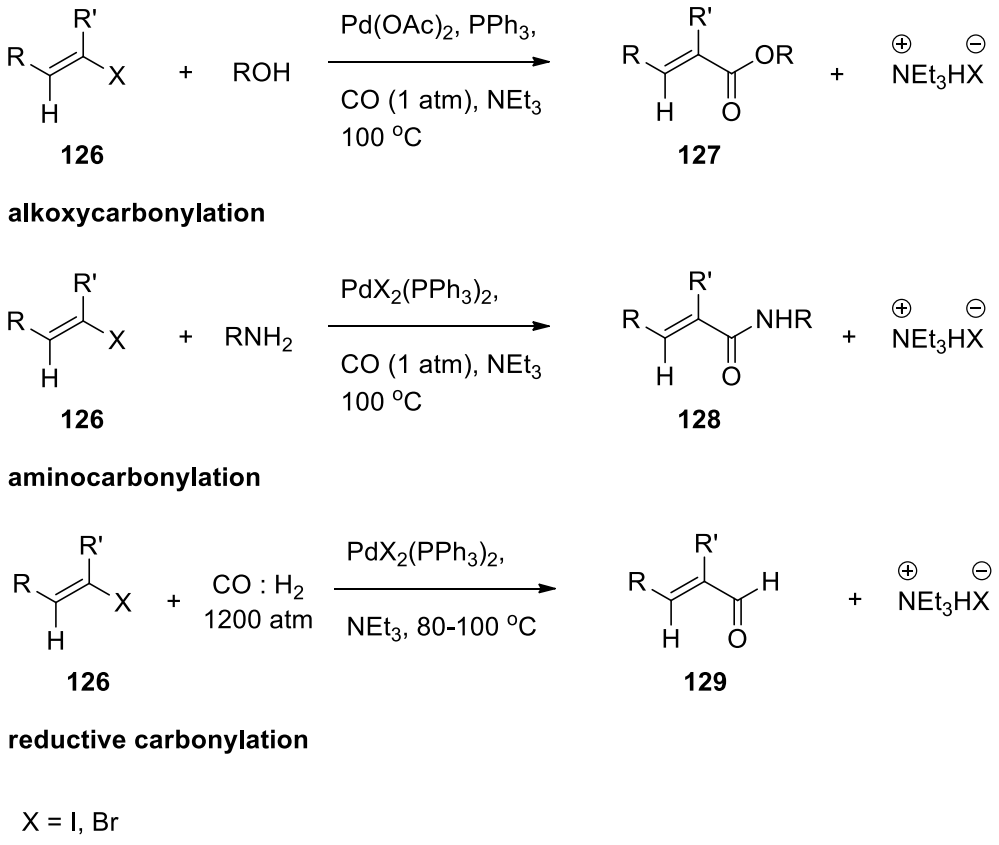


123 **124** **125**

Scheme 9: Hydroformylation process discovered by Roelen (R, R' = H, alkyl, aryl).[104]

Over the past 50 years, palladium-catalyzed cross coupling reactions have become increasingly important tools in synthetic chemistry, as evidenced by the awarding of the

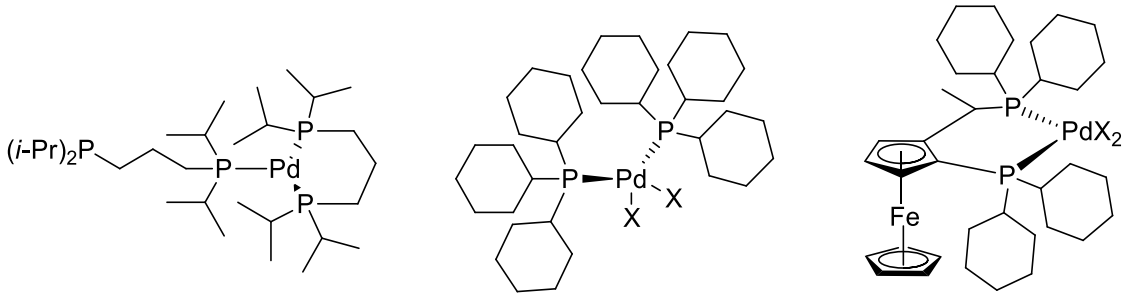
Nobel Prize to Heck, Negishi and Suzuki in 2010.[106] Palladium-catalyzed carbonylation, an example of an important of cross-coupling methodology, was first reported by Heck in 1974 (Scheme 10).[107] These seminal reports described the reaction of aryl and vinyl halides with carbon monoxide to form acylpalladium intermediates, which are then converted to carbonyl compounds by reaction with a nucleophile (Scheme 10).[107] Heck reported three variations of the carbonylation reaction: alkoxycarbonylation, aminocarbonylation, and reductive carbonylation that allowed access to three different potential products (**127**-**129**) (Scheme 10).[107]



Scheme 10: First report of palladium-catalyzed carbonylation by Heck.[107]

These reports established the applicability of aryl and vinyl bromides and iodides to this methodology, and demonstrated the requirement for a tertiary amine base. The standard catalyst system of a palladium (II) species with an excess of triphenylphosphine ligand, or a pre-formed palladium-triphenylphosphine catalyst was also established.[107]

The relatively mild conditions of these transformations led to their wide-spread application[108] and to studies aimed at expanding their potential utility. The applicability of alkoxy- and aminocarbonylation was extended to aryl and vinyl chlorides,[109] triflates,[110] tosylates,[111] and mesylates.[112] In order to overcome the energy barrier for oxidative insertion into some C-X bonds, ligand systems were designed to increase the electron density at palladium, examples of which are shown in Figure 26 (**130**-**132**).[109,113,114]



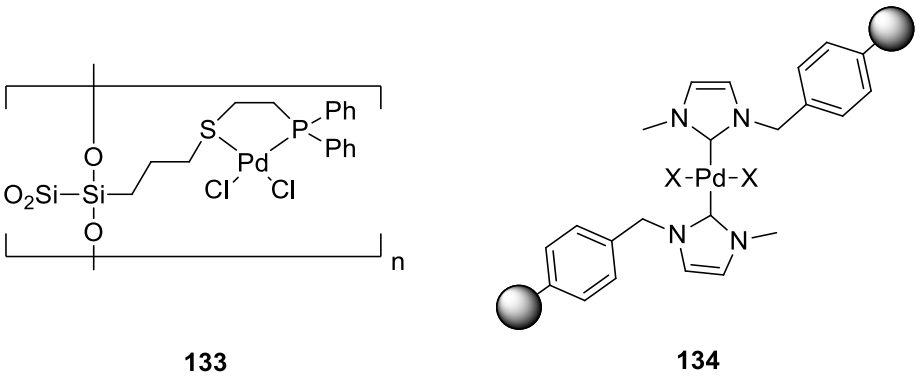
130 **131** **132**

Figure 26: Alternate catalyst systems designed to increase electron density at palladium.[109,113,114]

In an effort to eliminate the need for toxic, gaseous CO, it was shown that $Mo(CO)_6$ could act as a surrogate.[115] Stille demonstrated that Bu_3SnH could be

effectively used as a hydride donor in reductive carbonylation as an alternate to hydrogen gas.[116] Silanes such as Et_3SiH were also shown to be effective hydride sources.[117]

More recently, the focus of carbonylation research has shifted to creating a more efficient and environmentally benign catalyst system,[106(e)] leading to the development of re-usable, polymer supported catalysts, such as those shown in Figure 27 (**133**, **134**).[118] These catalysts can readily be filtered from the reaction mixture and reused many times, and they eliminate the need for the addition of external phosphine ligands.[118]



133 **134**

Figure 27: Re-usable, polymer supported carbonylation catalysts.[118]

Other transition metals have been shown to catalyze the carbonylation of aryl and vinyl halides, including nickel[107(a),119] and cobalt.[120] Carbonylation reactions catalyzed by nickel require higher catalyst loading and higher temperatures,[107(a),119] while catalysis with cobalt required simultaneous irradiation with light.[120] For these reasons, palladium catalysis remains the preferred means for the carbonylation of aryl and vinyl halides in academic and industrial settings.[108]

2.3.2 Mechanism

In his seminal report of palladium-catalyzed alkoxycarbonylation, Heck proposed two potential mechanisms, as shown in Figure 28.[107(a)] Although Heck considered both possible mechanisms, mechanism B was favoured, as it was known that organopalladium species can readily undergo CO insertion.[107(a)]

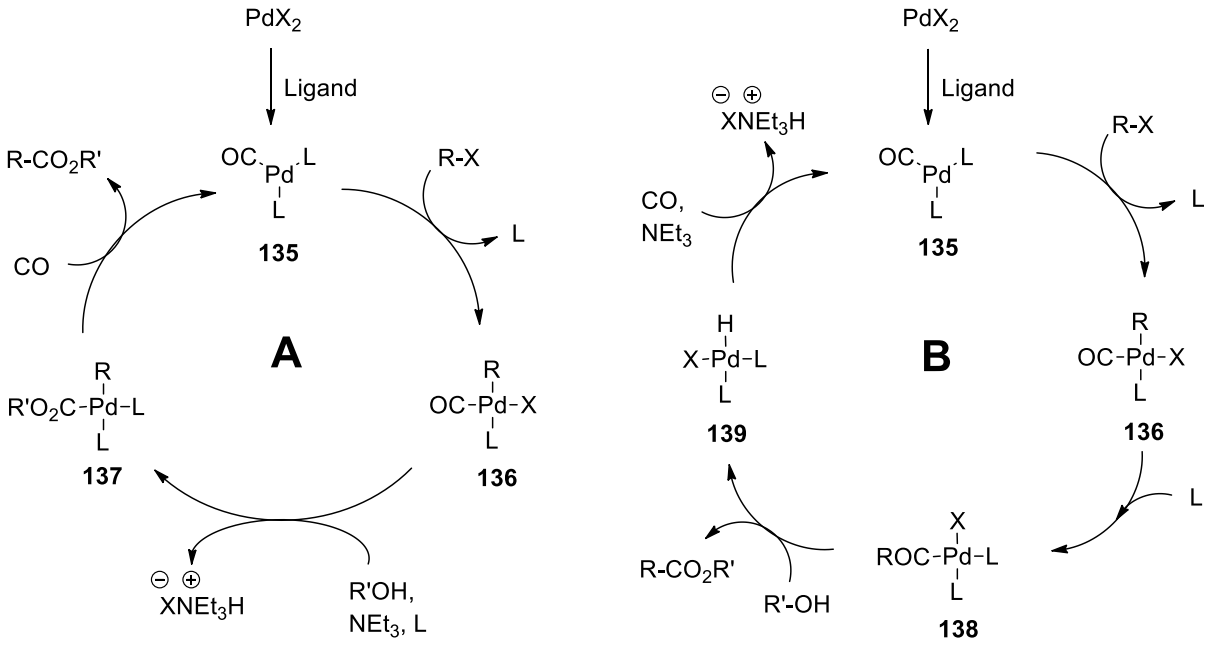


Figure 28: Mechanistic proposals made by Heck in 1972 (R = aryl/vinyl, L = ligand).[107(a)]

The primary difference between the two proposed mechanisms lies in the mode of attack of the nucleophile on the palladium-bound CO (**136**) and the subsequent reductive elimination in A.[107(a)] This is contrasted by the migratory insertion of CO in the organo-palladium species (**136**) followed by nucleophilic attack to form the product in B.[107(a)]

It is now recognized that CO ligands reduce the electron density at palladium, making it likely that the palladium (II) pre-catalyst is reduced to the active palladium (0)

species by the binding of ligands or solvent as opposed to the binding of CO (**135**) (Figure 28).[106(e),109] Oxidative insertion of palladium (0) to the carbon-halide bond of the substrate then occurs, forming an organo-palladium species of type **136** (Figure 28).[106(e),107(a)] The binding of CO follows oxidative insertion, and occurs rapidly for unhindered substrates, but can be rate limiting for hindered substrates.[121] Migratory CO insertion into the carbon-palladium bond then proceeds rapidly and irreversibly, even at room temperature, which precludes the possibility of mechanism A, as originally proposed by Heck.[107(a),109(a)] The steps following migratory insertion remain the subject of debate.

Yamamoto proposed a mechanism shown in Figure 29, in which migratory insertion was followed by nucleophilic attack at palladium to form an intermediate of type **142**.[122] This contrasted with the direct nucleophilic attack of the acyl-palladium species proposed by Heck (mechanism B) (Figure 28).[107(a),109(a)] For details on the research supporting this mechanism, see reference [122].

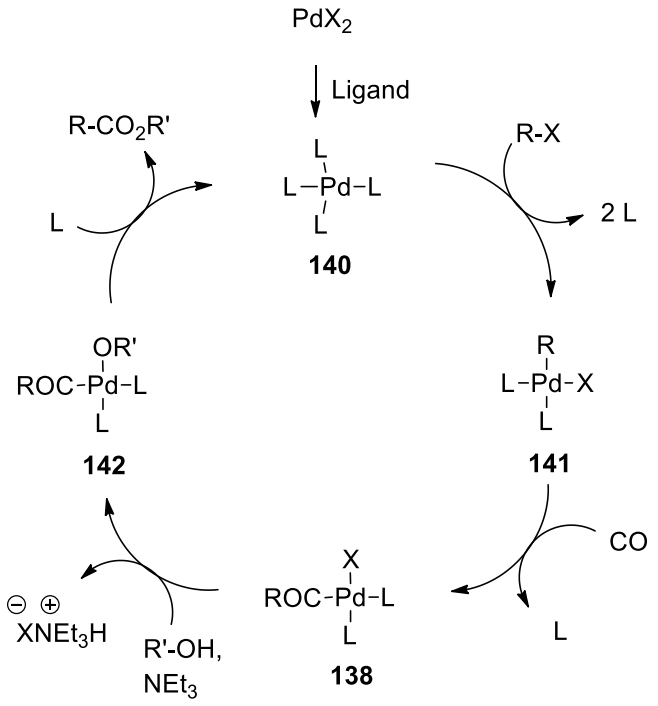


Figure 29: Carbonylation mechanism proposed by Yamamoto.[122]

Research by Hidai and Milstein however, supported the direct nucleophilic attack of the acyl-palladium species (**138**) as proposed by Heck.[109(a),123] For details on this research supporting this proposed mechanism, see references [109(a),123].

In the mechanism proposed by Yamamoto, product release occurs from complex **142** by reductive elimination,[122] and in the case of the direct nucleophilic attack, product release occurs as a result of base-assisted alcoholysis (or aminolysis) of the acyl-palladium species (**138**).[109(a)] Regardless of whether the direct nucleophilic attack mechanism is favoured, the overall mechanism for the palladium-catalysed carbonylation of aryl and vinyl (pseudo)halides can be generalized as shown in Figure 30.[106]

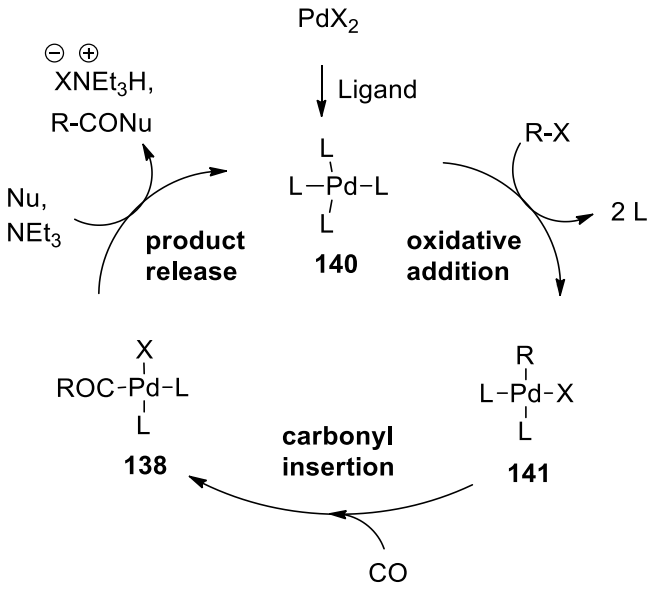


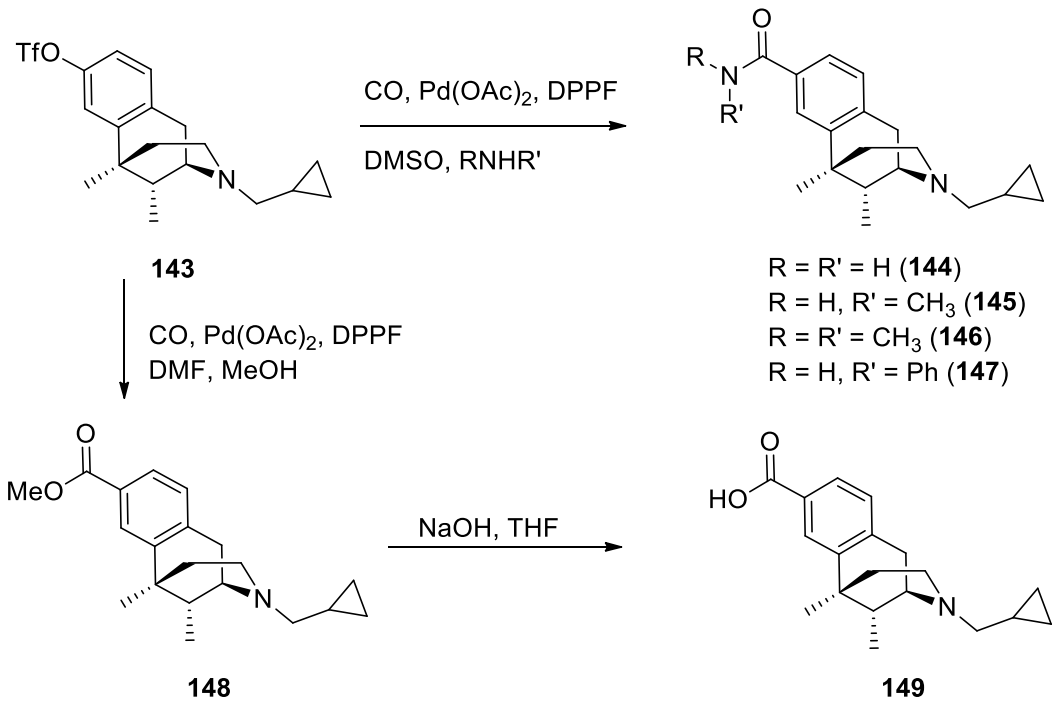
Figure 30: General mechanism for Pd-catalysed carbonylation of aryl/vinyl (pseudo)halides.[106]

2.3.3 Use of carbonylation chemistry in organic synthesis

Palladium-catalyzed carbonylation has become an extremely valuable tool in synthetic chemistry, because of its utility for the installation of various carbonyl functionalities and its functional group tolerance.[106,108] The use of this transformation in organic synthesis is ubiquitous, and the discussion here will serve only to highlight key examples of the application of alkoxycarbonylation, aminocarbonylation and reductive carbonylation.[106,108]

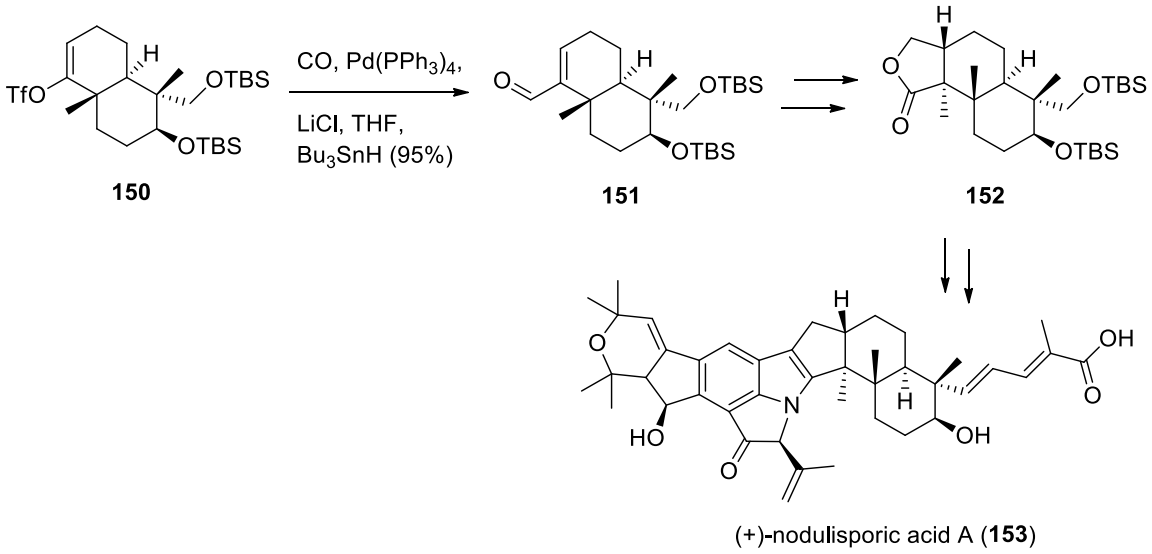
An excellent example of the versatility of palladium-catalyzed carbonylation is the synthesis of 8-carboxamidocyclazocine analogues (**144-149**) by Wentland (Scheme 11).[124] In this study, both alkoxy- and aminocarbonylation were applied in the late-stage introduction of ester and amide functionalities to 8-carboxamidocyclazocine analogues.[124] In this way, six different analogues (**144-149**) were synthesized from one common

intermediate (**143**) (Scheme 11).[124] These compounds were shown to have an unexpectedly high affinity for opioid receptors, and have contributed significantly to the understanding of the structure-activity relationship of this family of compounds.[124]



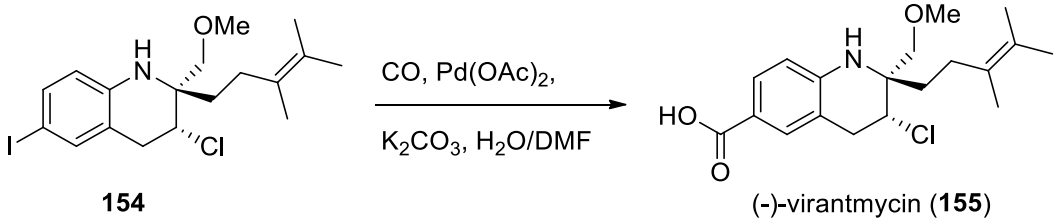
Scheme 11: Synthesis of 8-carboxamidocyclazocine analogues (**143-149**) by Wentland.[124]

Reductive carbonylation was applied in a "scalable" synthesis of lactone **152**, a key intermediate in the production of the potent insecticide (+)-nodulisporic acid (**153**) (Scheme 12).[125] This carbonylation reaction was readily performed on ten-gram scale in very high yield (Scheme 12).[125]



Scheme 12: Synthesis of lactone **152** by Smith.[125]

Pseudohalides such as triflates are commonly employed as carbonylation substrates in organic synthesis because of their ready availability from alcohols.[106,108,124,125] Halides are also commonly used, as was the case in the synthesis of the potent anti-viral compound (−)-virantmycin (**155**) by Kogen (Scheme 13).[126] In this particular carbonylation reaction, water acted as the nucleophile resulting in the production of the carboxylic acid moiety of (−)-virantmycin (**155**) (Scheme 13).[126]



Scheme 13: Synthesis of (−)-virantmycin (**155**) by Kogen.[126]

Although exceptionally brief, the above discussion serves to exemplify the versatility and the potential practical applicability of the palladium-catalyzed carbonylation of aryl and vinyl (pseudo)halides.

The following section will provide a discussion on alkylcyclohexenone natural products including their isolation, biological activity and their production by organic synthesis.

2.4 Alkylcyclohexenone natural products

One of the topics discussed in this thesis will be the chemoenzymatic synthesis of pleiogenone A (**14**) (Figure 31). The following section will provide a brief review of the isolation, biological activity and synthesis of various compounds of this type.

2.4.1 Isolation and biological activity

In the past 20 years, many hydroxylated cyclohexenone natural products have been isolated from various plant and fungal sources, representative examples of which are shown in Figure 31 (**14**, **156**-**165**).[17,127,128,129,130,131,132] Many of these compounds have interesting and potentially useful biological activity, leading to an interest in these compounds by medicinal and synthetic chemists.

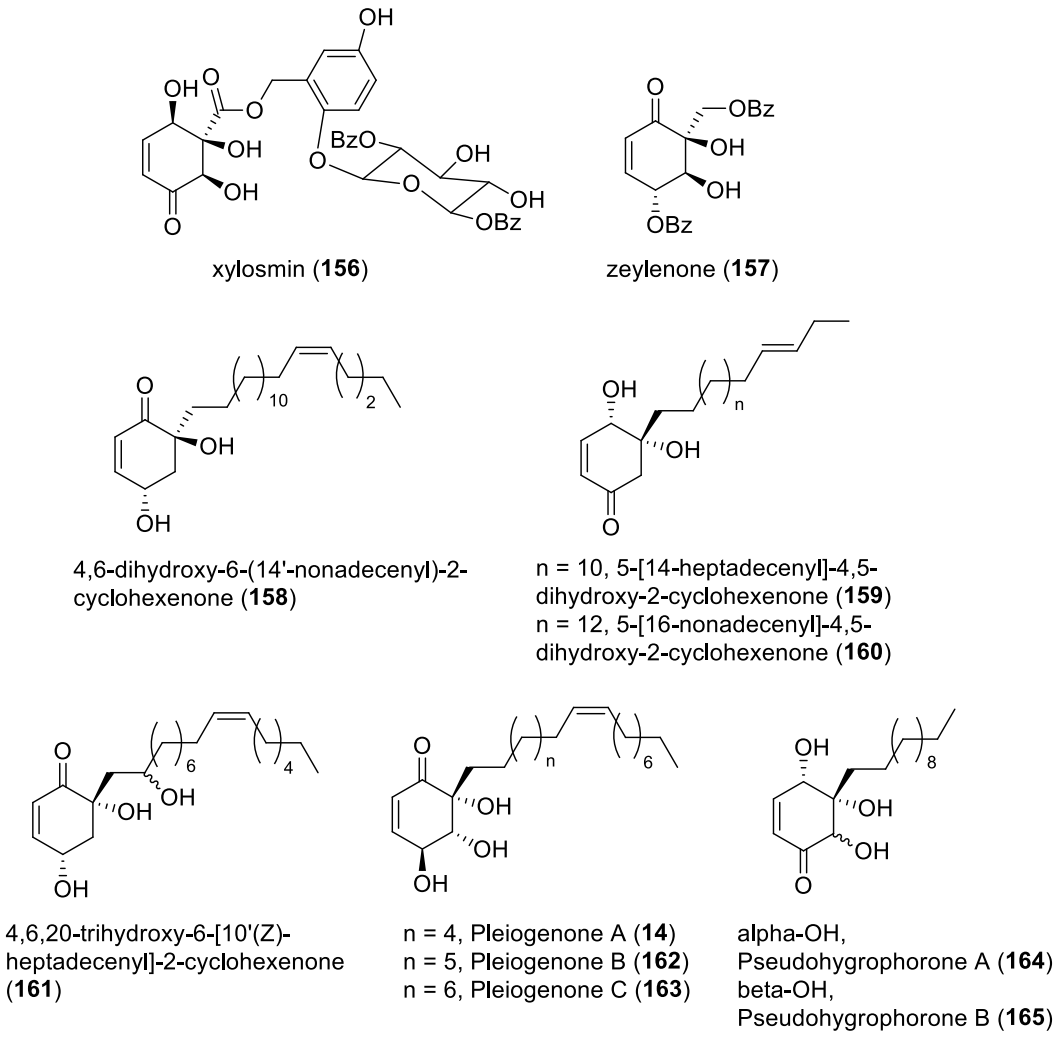


Figure 31: Examples of hydroxylated cyclohexenone natural products.[17,127-132]

The first of the compounds discussed here is xylosmin (**156**), which was isolated from *Xylosma flexuosum* in 1995 by Gibbons.[127(a)] Gibbons also proved the structure and absolute stereochemistry of xylosmin.[127(a)] In 2012, a series of closely related compounds were also identified, differing from xylosmin only in the extent and regioselectivity of their benzoylation.[127(b)] These compounds have been shown to inhibit the replication of dengue virus,[127(b)] as well as the growth of malaria parasites.[133]

Zeylenone (**157**) was isolated from *Uvaria grandiflora* by Liao in 1997, who also determined its structure and relative stereochemistry.[128(a)] Zeylenone (**157**) was shown to be a potent inhibitor of nucleoside transport in carcinoma cells,[128(a)] and was later shown to induce apoptosis in a variety of cancer cell lines.[134] Furthermore, zeylenone has recently been tested in animal studies as a potential local-delivery anti-tumour agent.[135] In 2001, the related compounds 3-*O*-debenzoylzeylenone and 2-*O*-benzoyl-3-*O*-debenzoylzeylenone as well as zeylenone (**157**) were isolated from *Uvaria purpurea*, and these compounds were all shown to inhibit root growth in *Lactuca sativa*.[128(b)]

Compound **158** was isolated from *Tapirira obtusa* by Cordell in 2001, who also determined the structure and relative stereochemistry of this compound.[129] Cordell proposed that **158** was a biogenetic precursor to dermatitis-causing *n*-alkylphenols known to be produced by plants in this family, but did not report any biological activity data.[129] Compounds **159** and **160** were isolated from *Lannea edulis* by Hostettmann in 2003.[130] Hostettmann also determined the structure of these compounds, as well as their absolute configuration, through the use of the Mosher ester method.[130,136] Compounds **159** and **160** did not demonstrate any anti-fungal or anti-bacterial activity.[130] More recently, Roumy and Fabre isolated compound **161** from *Tapirira guianensis*, and determined its structure and relative configuration.[131] Compound **161** was shown to have anti-plasmodial and anti-bacterial properties, which supported the traditional use of *Tapirira guianensis* in the treatment of external infections.[131]

The pleiogenone family of compounds (**14**, **162**, **163**) was isolated in 2015 by Kingston.[17] After determining the structure of these compounds by nuclear magnetic resonance (NMR) and mass spectrometry (MS) analysis, Kingston determined the

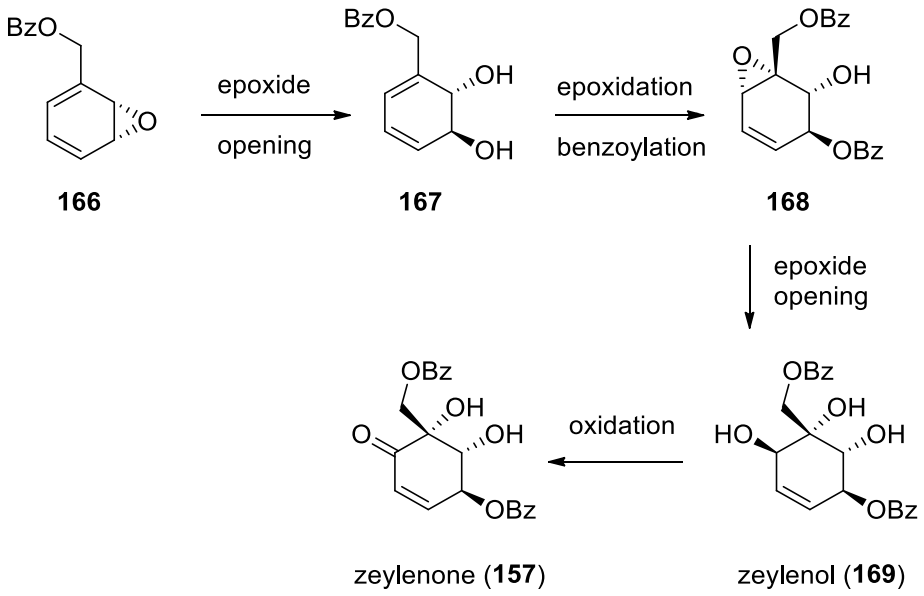
absolute configuration of pleiogenones A-C (**14**, **162**, **163**).[17] The anti-proliferative properties of pleiogenones A-C (**14**, **162**, **163**) were tested against an ovarian cancer cell line, and all three compounds exhibited moderate activity (IC_{50} = 0.7-0.8 μM).[17]

The pseudohygrophorones (**164**, **165**) were isolated in 2016 by Arnold,[132] from the fruiting bodies of the basidiomycete *Hygrophorus abieticola*. The pseudohygrophorones A and B were the first hydroxylated cyclohexenone natural products to be isolated from fungal sources. Arnold also determined the structure as well as the relative and absolute stereochemistry of these compounds. Pseudohygrophorone A and B (**164**, **165**) were shown to inhibit the growth of plant pathogenic fungi, with efficacy comparable to that of commercial pesticides.[132]

2.4.2 Biosynthesis

Although none of the compounds discussed in the section have been specifically studied in the context of their biosynthesis, proposals have been made as to the biosynthetic pathway towards hydroxylated cyclohexene natural products. The example of the biosynthesis of zeylenone (**169**) is given in Scheme 14.

The biosynthesis of compounds of this type was first addressed by Ganem, who stated that the pathway likely occurs through the initial formation of epoxide **166**, derived from benzyl benzoate (Scheme 14).[137,138] Opening of this epoxide would lead to *trans*-diol **167**, a motif which has been identified in compounds isolated from plants of the *Uvaria* genus.[139] A second epoxidation and benzoylation would afford intermediate **168**, which could be converted to zeylenol (**169**) though epoxide opening.[100,137,138] The oxidation of zeylenol (**169**) would afford zeylenone (**157**) (Scheme 14).



Scheme 14: Proposed biosynthetic pathway for zeylenol (**169**) and zeylenone (**157**).[100,138,139]

Because many hydroxylated cyclohexene natural products have been isolated in both enantiomeric forms, including zeylenol (**169**) and zeylenone (**157**) congeners,[100] proposals have been made to account for the biosynthesis of both enantiomers. The first proposal stated that epoxide **166** can racemize through the formation of oxepin **170** (Figure 32).[140] The existence of the enantiomeric form of epoxide **166** would lead to the enantiomer of zeylenol (**169**) and zeylenone (**157**) through the biosynthetic scheme outlined in Scheme 14. The second proposal stated that neighbouring-group assistance from the ester moiety of epoxide **166** can lead to the opening of the epoxide at C-2 (**171**), as opposed to C-3 opening shown in Scheme 14.[141] This process would lead to the production of the enantiomer of *trans*-diol **167**, and therefore to the eventual biosynthesis of the enantiomeric forms of hydroxylated cyclohexene natural products.

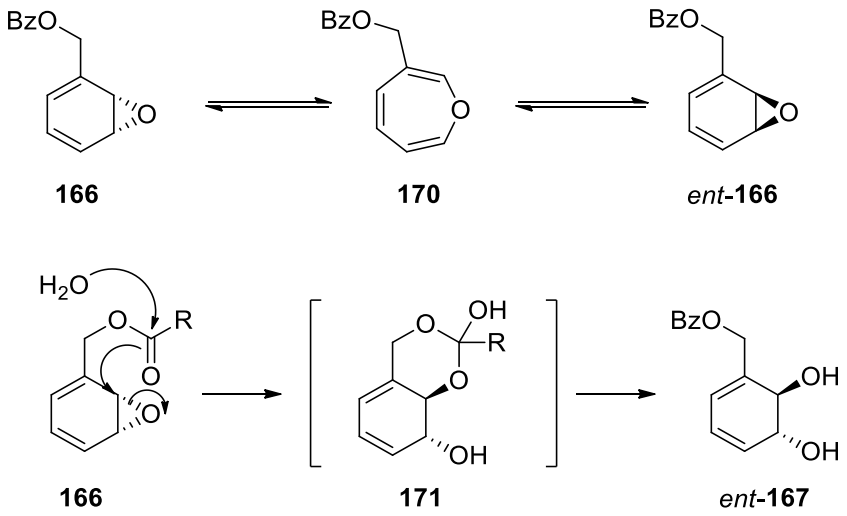


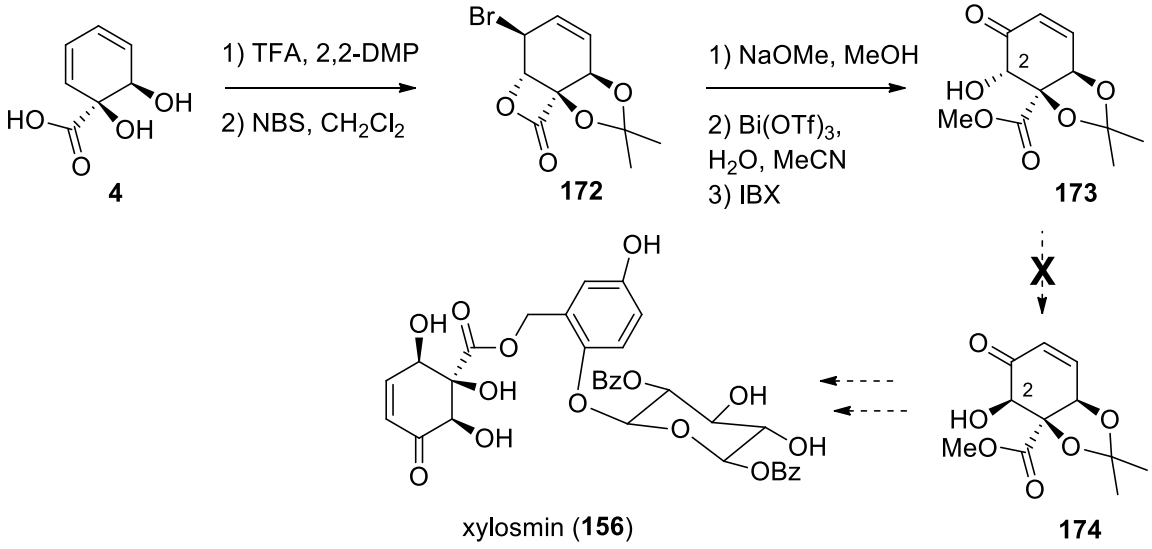
Figure 32: Proposed transformations in hydroxylated cyclohexene biosynthesis.[140,141]

These biosynthetic proposals can be used to account for the hydroxylation observed in many hydroxylated cyclohexene natural products including xylosmin (**156**), zeylenone (**157**) and pleiogenones A-C (**14**, **162**, **163**). The biosynthetic origin of other moietites such as the alkenyl groups present in the pleiogenenones, the pseudohydrophorones and compounds **158-161**, remains unknown.

2.4.3 Syntheses

Of the compounds discussed in this section, synthetic approaches have been reported for xylosmin (**156**) and zeylenone (**157**). Inspired by recent reports of the anti-malarial and anti-viral properties of compounds of this type,[127(b),133] the first approach towards the synthesis of xylosmin (**156**) was undertaken by Hudlicky in 2015.[142] This synthetic approach was focused on the production of the hydroxylated cyclohexenone fragment of xylosmin (**156**), as the phenolic and saccharide moieties can be readily installed at a later stage. The enzymatic metabolite, *ipso*-diol **4** was used as the starting

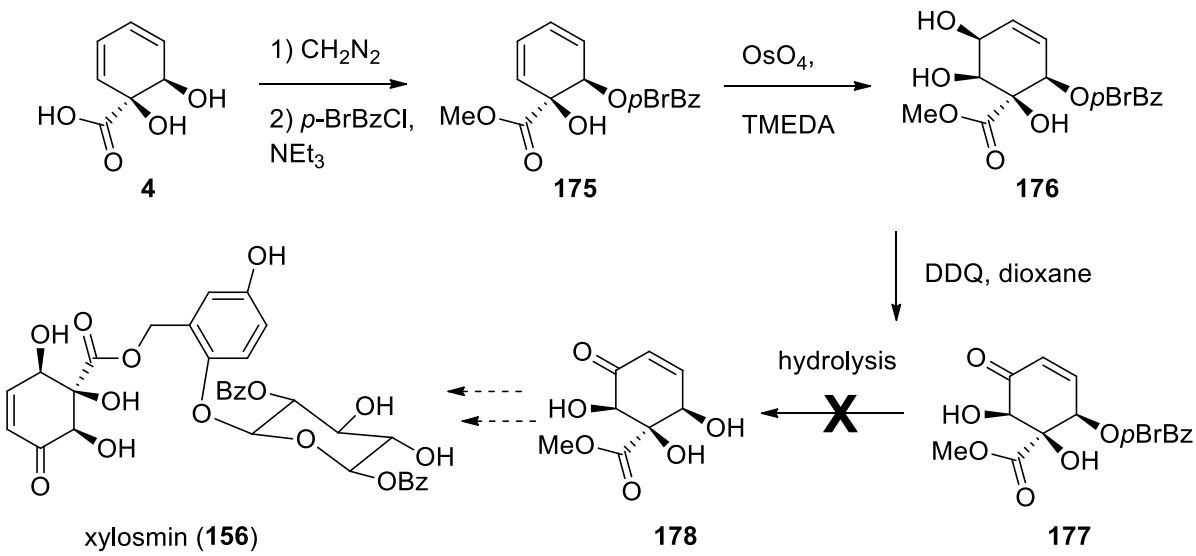
material, as it contains some of the desired functionality present in xylosmin (**156**). In the first approach towards xylosmin, *ipso*-diol **4** was converted to lactone **172**, through a process developed by Myers (Scheme 15).[93] Lactone **172** was then opened with sodium methoxide to isolate an epoxide which was selectively opened with bismuth triflate and oxidized with 2-iodoxybenzoic acid (IBX) to afford enone **173**. Unfortunately, all attempts to invert the stereochemistry of the C-2 hydroxyl group, either through enolization/epimerization or Mitsunobu reaction, failed.



Scheme 15: First approach towards xylosmin (**156**) by Hudlicky.[142]

The synthetic strategy was then altered to involve a key hydrogen-bond directed Donohoe dihydroxylation[143] of ester **175** (prepared in two steps from *ipso*-diol **4**), to ensure the correct C-2 stereochemistry. Following dihydroxylation, the selective oxidation of the allylic alcohol in triol **176** was affected with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (Scheme 16). Attempts to hydrolyse the *para*-bromo benzoyl

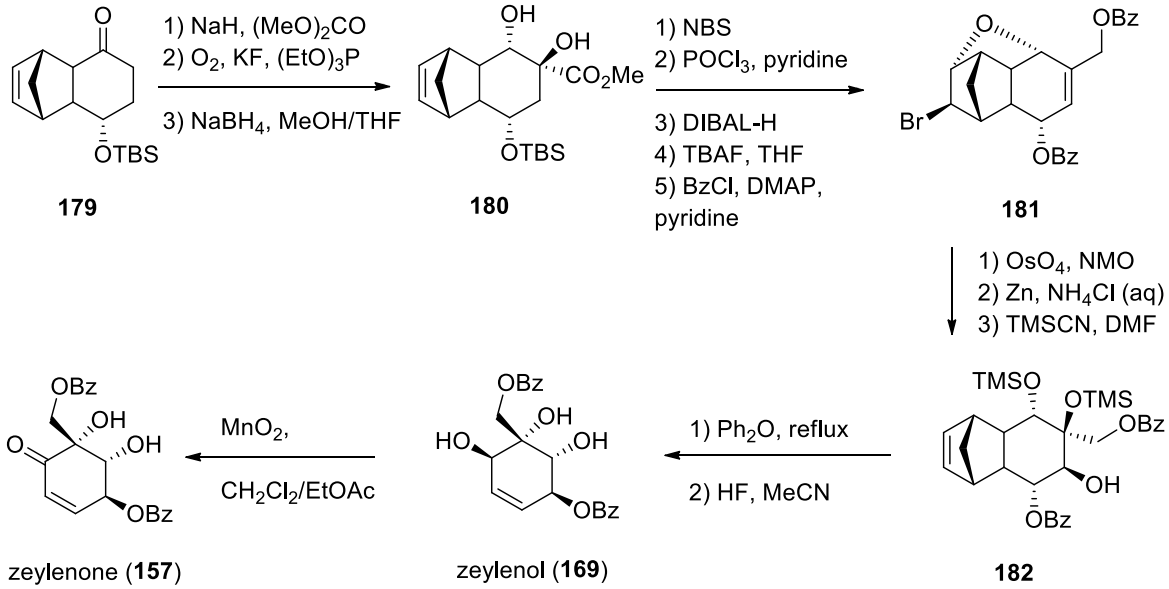
ester moiety were not successful, however this report constitutes the first synthetic approach towards xylosmin (**156**).



Scheme 16: Second synthetic approach towards xylosmin (**156**) by Hudlicky.[142]

The first synthesis of zeylenone (**157**) was reported by Ogawa in 1999.[144] This synthesis used silyl ether **179**, previously prepared by Ogasawara,[145] as the starting material (Scheme 17). Methoxycarbonylation of **179** and the subsequent hydroxylation and reduction all occurred from the convex face of the molecule, affording diol **180**. Bromo-ether formation was then effected with NBS, and was followed by a four-step sequence to produce benzoate **181**. Upjohn dihydroxylation[146] was performed after the benzoylation step, a design which allowed for the benzoylation of only the required positions. Upjohn dihydroxylation[146] occurred exclusively on the convex face of the molecule, and was followed by regeneration of the cyclopentene olefin and silyl protection of the resulting triol to afford olefin **182**. The hetero Diels-Alder reaction of

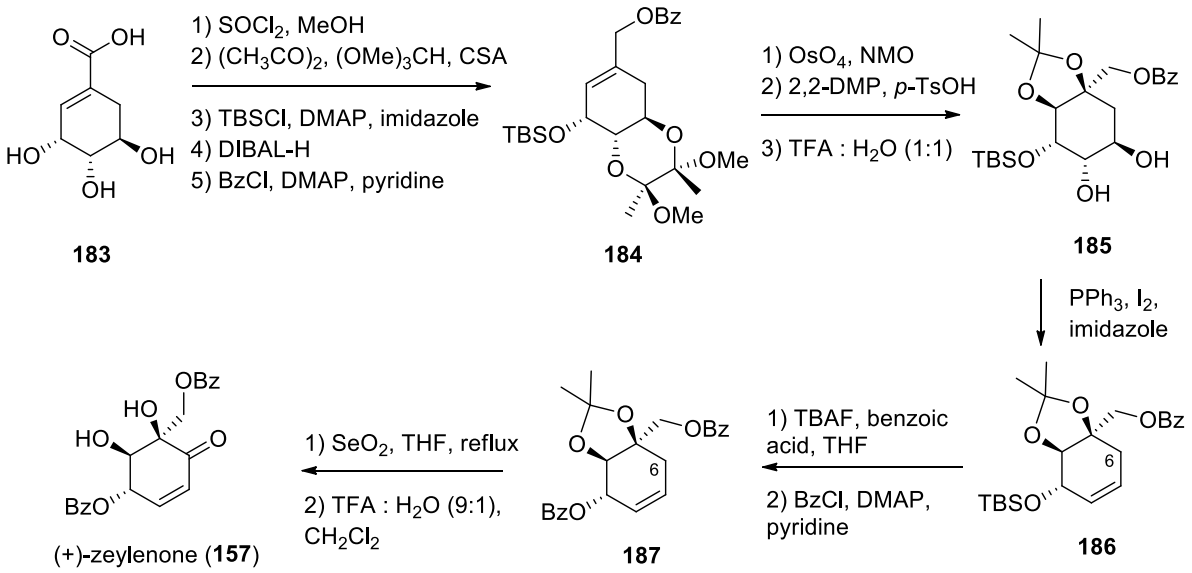
olefin **182** and subsequent silyl deprotection furnished zeylenol (**169**), which was then oxidized to zeylenone (**157**).



Scheme 17: Synthesis of zeylenol (**169**) and zeylenone (**157**) by Ogawa.[144]

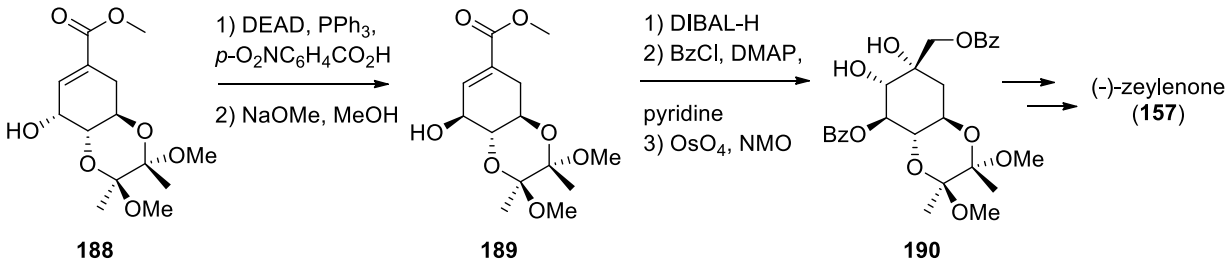
The second synthesis of zeylenone (**157**), published in 2004 by Liu and Xu,[147] reported the production of the enantiomer of the natural product, (+)-zeylenone (**157**), and was later expanded to the synthesis of the natural enantiomer (−)-zeylenone (**157**).[148] This approach employed a chiral pool strategy, with shikimic acid (**183**) as the starting material (Scheme 18). In a sequence of five steps, shikimic acid (**183**) was converted to benzoate **184**, the desired substrate for the key Upjohn dihydroxylation step. The stereoselective Upjohn dihydroxylation occurred on the less hindered β-face of benzoate **184**, and was followed by acetonide protection of the resultant diol and selective deprotection to afford diol **185**. The diol moiety was then converted to the corresponding

alkene, producing olefin **186**. All attempts to oxidize olefin **186** at C-6 with the goal of introducing the enone functionality failed, causing the authors to alter the order of operations and prepare benzoate **187**. The C-6 oxidation of benzoate **187** was successful, and was followed by acetonide deprotection to afford (+)-zeylenone (**157**).



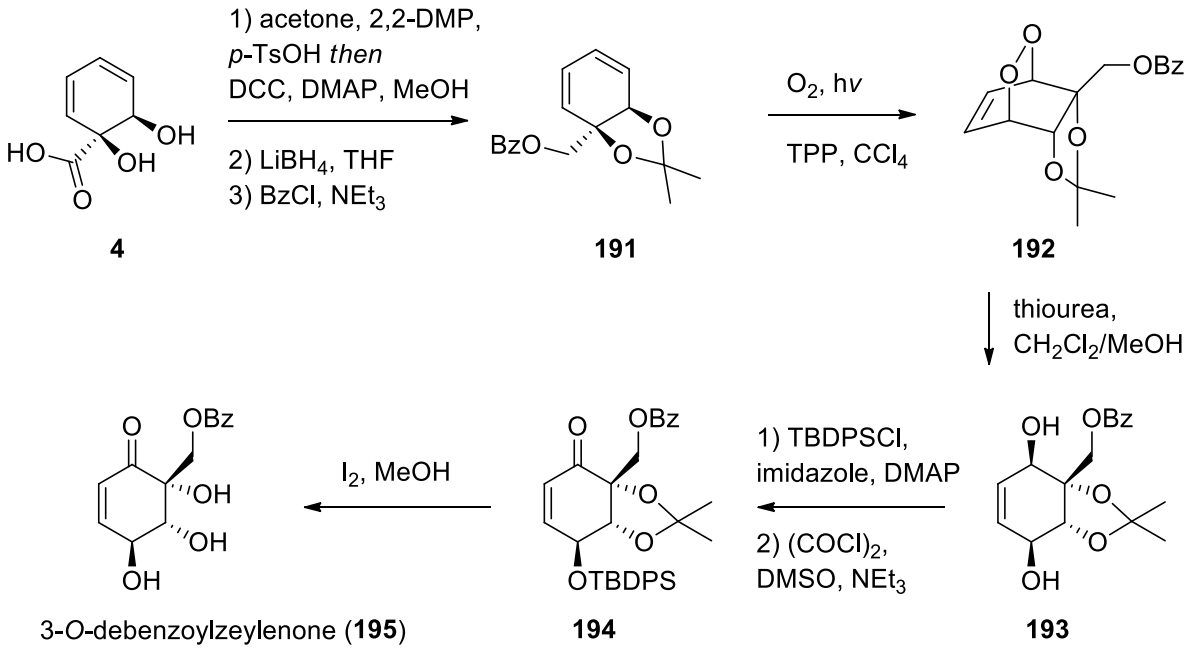
Scheme 18: Synthesis of (+)-zeylenone (**157**) by Liu and Xu.[147]

In 2006, this synthetic strategy was adjusted in an enantiodivergent manner in order to produce the natural enantiomer, (−)-zeylenone (**157**) from the same starting material.[148] The enantiodivergence was achieved by the application of the Mitsunobu reaction to ester **188**, a known intermediate from the previous synthesis, to afford alcohol **189** (Scheme 19). This stereochemical inversion also altered the steric influence on the key Upjohn dihydroxylation, resulting in stereoselective dihydroxylation from the α-face (**190**). In this way, the required stereochemistry was achieved in the synthesis of natural (−)-zeylenone (**157**).



Scheme 19: Enantiodivergent synthesis of (−)-zeylenone (**157**) by Liu and Xu.[148]

The most recent approach to zeylenone congeners was reported by Lewis in 2012.[100] This report describes the synthesis of zeylenol (**169**), as well as two natural zeylenol isomers, and the natural zeylenone congener 3-*O*-debenzoylzeylenone (**195**). As in Hudlicky's approach towards xylosmin, the enzymatic metabolite *ipso*-diol **4** was used as a starting material (Scheme 20). The key transformation in this synthesis is the cycloaddition of benzoate **191** (prepared in three steps from *ipso*-diol **4**) with singlet oxygen, which occurred exclusively on the less hindered β-face of the molecule to afford endoperoxide **192**. Reduction of the endoperoxide moiety led to diol **193**, which was selectively protected and the free alcohol oxidized to afford enone **194**. The acetonide and silyl protecting groups were then simultaneously deprotected with iodine in methanol to afford 3-*O*-debenzoylzeylenone (**195**), completing the synthesis in a total of eight steps and in an overall yield of 15%.[100]



Scheme 20: Synthesis of 3-*O*-debenzoylzeylenone (**195**) by Lewis.[100]

The primary challenge in the syntheses discussed in this section is the introduction of the many hydroxyl moieties in the correct stereochemical configuration. Some of the strategies discussed here addressed this challenge in part by applying a chiral pool approach, while others used enzymatic dihydroxylation. In order to introduce the additional hydroxyl moieties in a stereoselective manner, Hudlicky, Ogawa as well as Liu and Xu employed osmium-mediated dihydroxylation, while Lewis used a stereoselective cycloaddition with singlet oxygen.[142,144,145,148] This concludes the historical overview of key areas that will be discussed in this thesis.

3. Results and Discussion

3.1 Introduction

With the review of dioxygenase enzymes, carbonylation chemistry and hydroxylated cyclohexenone natural products complete, the key projects of this dissertation will be discussed. These are, in order;

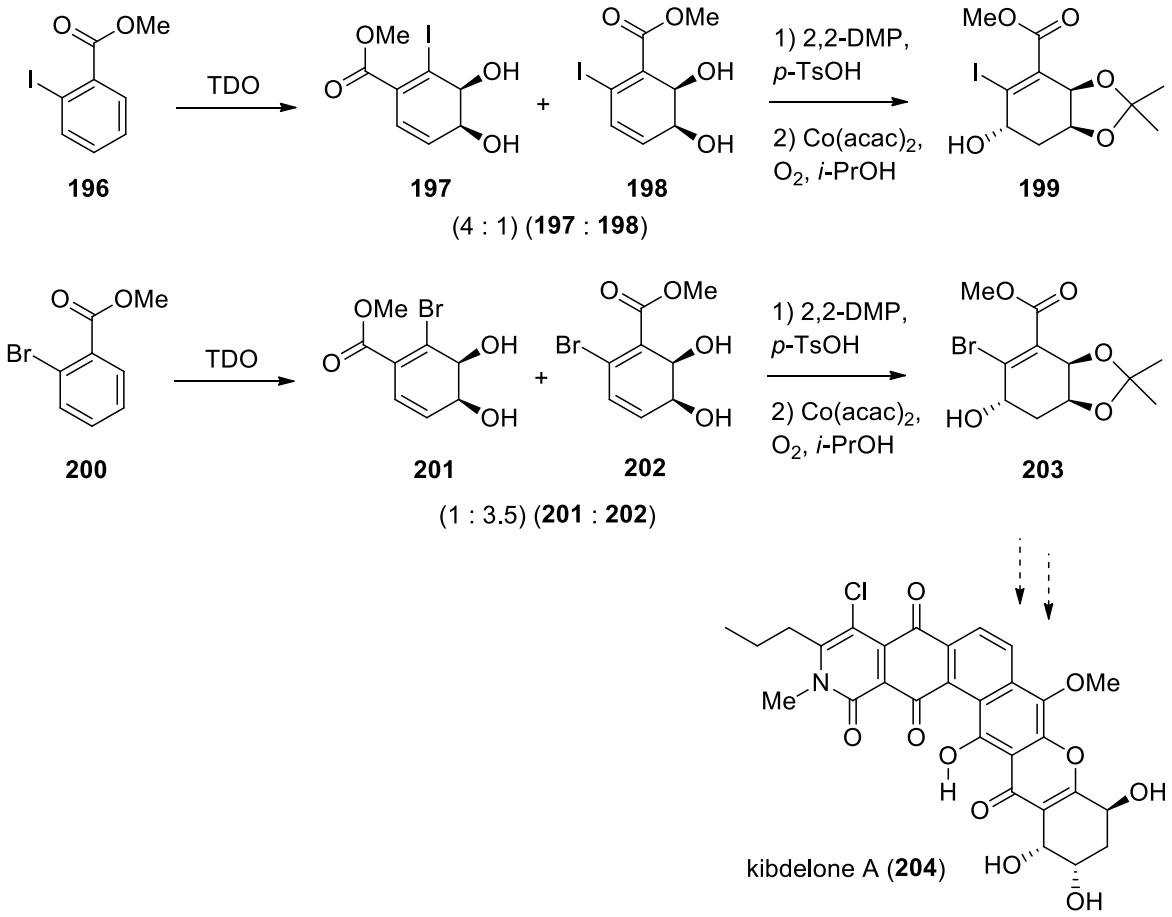
1) Investigation of TDO selectivity and substrate scope
2) Carbonylation of halo *cis*-cyclohexadiene diols to the corresponding carboxylates
3) Approaches towards the synthesis of tetrodotoxin
4) Synthesis of pleiogenone A

3.2 Investigation of TDO selectivity and substrate scope

3.2.1 Dihydroxylation of *ortho*-halo benzoates

In 2011, Hudlicky reported the formal synthesis of kibdelone C and its congeners, through the production of intermediate **199** (Scheme 21).[149] This key intermediate was synthesized in just two steps from diol **198**, as compared to Porco's 14-step synthesis of this compound from glyceraldehyde.[150] Although this reported process demonstrated a high degree of brevity, it was limited by the fact that diol **198** was produced as the minor isomer from the TDO-mediated enzymatic dihydroxylation of methyl 2-iodobenzoate (**196**). This report led to a collaboration between Hudlicky and Porco in the synthesis of kibdelone A. In the course of this collaboration, it was found the brominated analogue

203, made from diol **202**, could be used as a starting material (Scheme 21).[151] In agreement with Boyd's model for the regioselectivity of TDO-mediated dihydroxylation,[57] (Section 2.2.2.2) the dihydroxylation of methyl 2-bromobenzoate (**200**) led to the preferential production of the desired regioisomer **202**, which significantly increased the yield of the overall process.



Scheme 21: Use of metabolites **198** and **202** in the synthesis of kibdelone congeners.[149,151]

In 2012, the TDO-mediated dihydroxylation of the full series of methyl 2-halobenzoates was studied, as discussed in section 2.2.2.2 (Figure 19).[69] The ratio of regioisomeric products was investigated, and the findings provided further support to Boyd's model,[57] with sterically larger halogen substituents demonstrating a greater directing effect on the dihydroxylation (Figure 19).[69]

In order to expand this study, the steric size of the ester substituent was varied in addition to the halogen (Figure 33). The goals of this study were to measure the effect of differential steric size on the ratio of regioisomeric products, and to provide greater predictive power to Boyd's model[57] in planning future synthetic sequences.

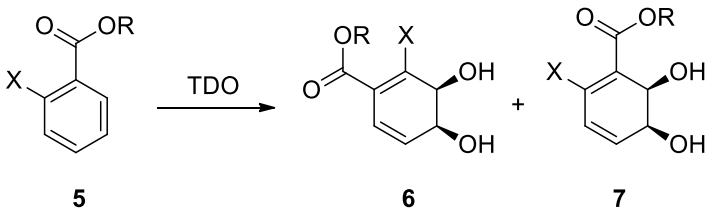
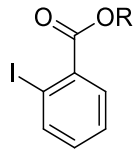
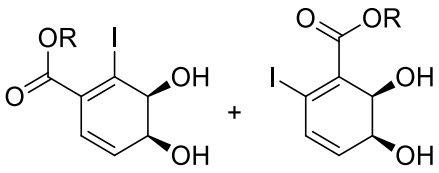


Figure 33: TDO-mediated dihydroxylation of *ortho*-halobenzoates (**5**).

To investigate the regioselectivity of the dihydroxylation of *ortho*-halo benzoates, large-scale (10 L) fermentation cultures of *E. coli* JM109 (pDTG601A)[54] were grown according to reported procedures.[14(m)] The cultures were grown to maximum optical density, and fed with the aromatic substrate until consumption of the substrate was no longer observed (monitored as a function of O_2 consumption). The ratio of regioisomeric products was determined by ^{1}H NMR analysis of the crude fermentation extract, and yields were determined after purification of the mixture. As the regioisomers proved

difficult to separate, the purification of these compounds was performed with the use of preparative high-pressure liquid chromatography (HPLC). The results of this study are reported in Tables 4-7.

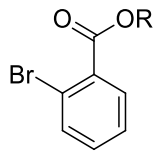
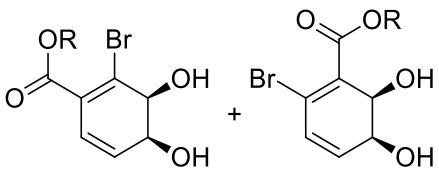
Table 4: Results of the TDO-mediated dihydroxylation of 2-iodobenzoates.

		
R =	Ratio of products[a]	Yield (g/L)[b]
methyl (**196**)	4 (**197**) : 1 (**198**)[69]	0.33[69]
ethyl (**205**)	1 (**210**) : 1 (**211**)	0.04
n-propyl (**206**)	1 (**212**) : 2 (**213**)	trace
allyl (**207**)	1 (**214**) : 4 (**215**)	0.01
propargyl (**208**)	1 (**216**) : 12 (**217**)	0.07
t-butyl (**209**)	-	-

[a] determined by H NMR analysis of crude fermentation extract;
[b] yield of combined mixture

The initial results, obtained with 2-iodobenzoates (Table 4), provided support for Boyd's model.[57] As is evident from table 4, as the steric size of the ester substituent increased, its directing effect on the enzymatic dihydroxylation also increased. Furthermore, a general trend was observed that as the size of the ester substituent increased, the overall yield of the fermentation decreased. This result was expected based on previous reports.[66]

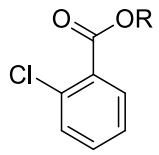
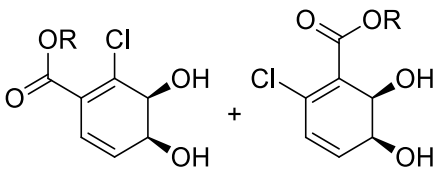
Table 5: Results of the TDO-mediated dihydroxylation of 2-bromobenzoates.

		
R =	Ratio of products[a]	Yield (g/L)[b]
methyl (**200**)	1 (**201**) : 3.5 (**202**)[69]	0.40[69]
ethyl (**218**)	1 (**223**) : 3 (**224**)	0.30
n-propyl (**219**)	1 (**225**) : 4 (**226**)	trace
allyl (**220**)	1 (**227**) : 10 (**228**)	0.02
propargyl (**221**)	1 (**229**) : 48 (**230**)	0.35
t-butyl (**222**)	-	-

[a] determined by H NMR analysis of crude fermentation extract;
[b] yield of combined mixture

The results obtained with 2-bromobenzoates (Table 5) were similar to those observed with 2-iodobenzoates (Table 4), further supporting Boyd's model.[57] The yields for fermentations with 2-bromobenzoates were generally higher than those observed with 2-iodobenzoates, as expected from previous reports.[66]

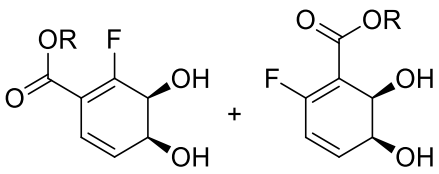
Table 6: Results of the TDO-mediated dihydroxylation of 2-chlorobenzoates.

		
R =	Ratio of products[a]	Yield (g/L)[b]
methyl (**231**)	1 (**237**) : 4 (**238**)[69]	0.33[69]
ethyl (**232**)	1 (**239**) : 3.5 (**240**)	0.04
n-propyl (**233**)	1 (**241**) : 20 (**242**)	trace
allyl (**234**)	1 (**243**) : 7 (**244**)	0.02
propargyl (**235**)	3 (**245**) : 97 (**246**)	0.30
t-butyl (**236**)	-	-

[a] determined by H NMR analysis of crude fermentation extract;
[b] yield of combined mixture

The results obtained with 2-chlorobenzoates (Table 6) were similar to those previously obtained (Tables 4 and 5), with respect to the general trends in regioselectivity and in yield. Despite the smaller steric size of the chlorine substituent relative to bromine, the lower yields observed with 2-chlorobenzoates relative to 2-bromobenzoates, were expected based upon previous results.[152]

Table 7: Results of the TDO-mediated dihydroxylation of 2-fluorobenzoates.

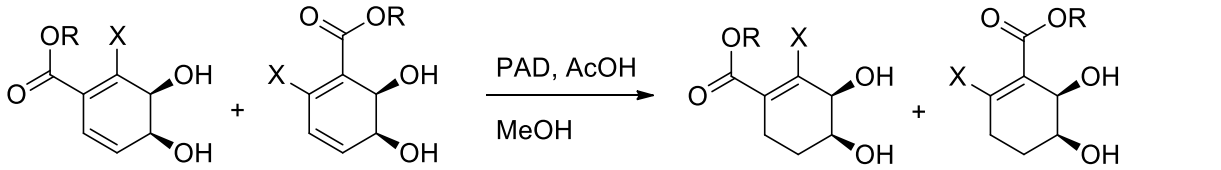
		
R =	Ratio of products[a]	Yield (g/L)[b]
methyl (**247**)	- (**253**) : 100 (**254**)[69]	0.33[69]
ethyl (**248**)	- (**255**) : 100 (**256**)	0.04
n-propyl (**249**)	- (**257**) : 100 (**258**)	trace
allyl (**250**)	- (**259**) : 100 (**260**)	0.02
propargyl (**251**)	- (**261**) : 100 (**262**)	0.30
t-butyl (**252**)	-	-

[a] determined by H NMR analysis of crude fermentation extract;
[b] yield of combined mixture

Consistent with previous findings,[69] 2-fluorobenzoates yielded only one regioisomer upon TDO-mediated dihydroxylation, irrespective of the size of the ester substituent (Table 7). Furthermore, significantly higher yields were obtained using 2-fluorobenzoates relative to the use of the other halogenated substrates.

As the diene diol metabolites (**210**-**217**, **223**-**230**, **239**-**246**) were found to be somewhat labile, these compounds were characterized by ^{1}H NMR, IR and optical rotation before being converted to their partially reduced derivatives (**263**-**271**) (Scheme 22). Fluorinated metabolites (**254**, **256**, **260**, **262**) displayed significantly greater levels of stability and were characterized fully. Reduced derivatives **263**-**271** had significantly increased stability, and were fully characterized. Because of the hydrogenation of the

allyl and propargyl ester moieties, all allyl and propargyl substituted metabolites converged to reduced derivatives **267**, **269** and **271** (Scheme 22). Metabolites **212**-**214**, **216**, **225**-**227**, **229**, **241**-**243**, **245** were not produced in quantities significant enough to facilitate characterization.



X = I (**210**), Br (**223**), Cl (**239**)
R = Et

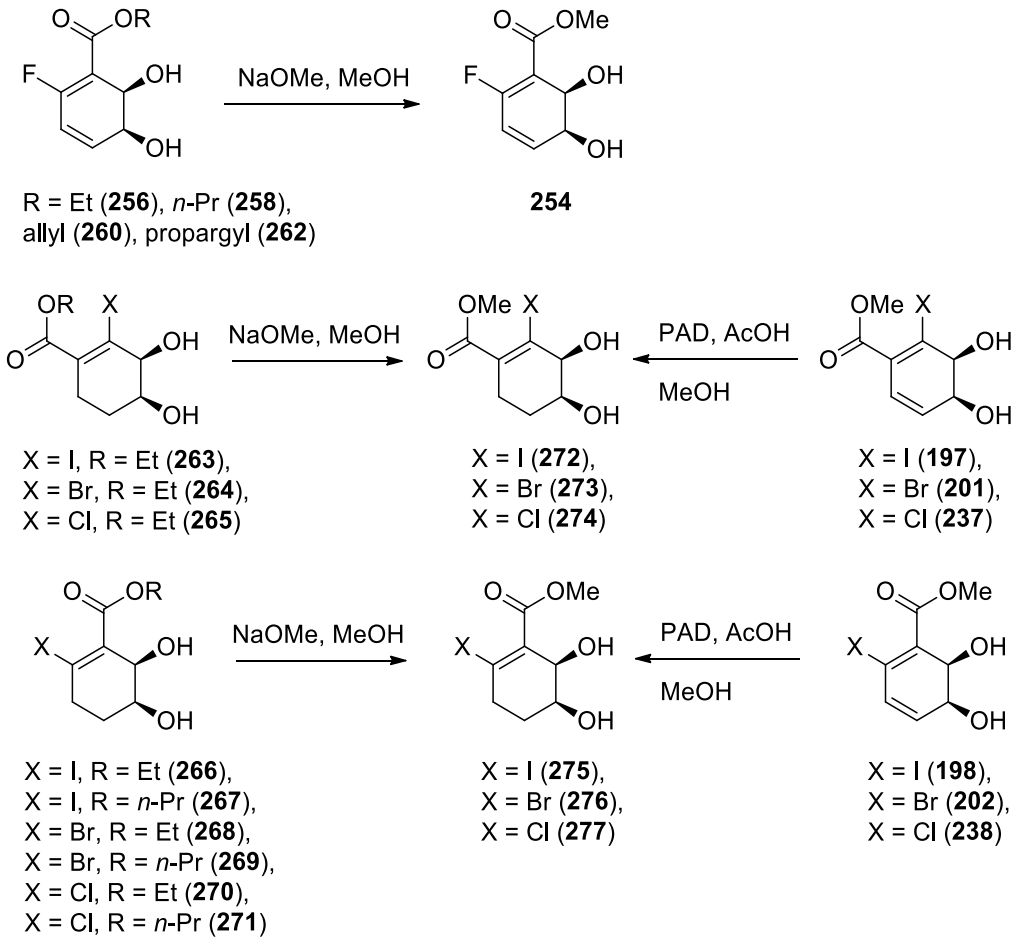
X = I (**211**, **215**, **217**), Br (**224**, **228**, **230**), Cl (**240**, **244**, **246**)
R = Et, allyl, propargyl

X = I, R = Et (**263**),
X = Br, R = Et (**264**),
X = Cl, R = Et (**265**)

X = I, R = Et (**266**),
X = I, R = *n*-Pr (**267**),
X = Br, R = Et (**268**),
X = Br, R = *n*-Pr (**269**),
X = Cl, R = Et (**270**),
X = Cl, R = *n*-Pr (**271**)

Scheme 22: Partial hydrogenation of diol metabolites to facilitate full characterization.

In order confirm the absolute stereochemistry in the new metabolites, all of the fluorinated derivatives were converted to the corresponding methyl ester **254** (Scheme 23), the absolute stereochemistry of which has been previously published.[69] In the case of the iodo, bromo, and chloro compounds, their reduced derivatives (**263**-**271**) were converted to their corresponding methyl esters (**272**, **273**) (Scheme 23). The partially reduced methyl ester metabolites (**272**, **273**) were matched by ^{1}H NMR, melting point, and optical rotation to their corresponding compounds previously prepared from metabolites **198**-**201**, **237**, **238**.[69]

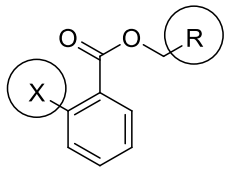


Scheme 23: Derivatization of metabolites for the confirmation of absolute stereochemistry.[69]

An interesting trend emerged when examining the isomeric ratios associated with the propargyl compounds. In the case of iodo, bromo, and chloro compounds, the propargyl ester substituent was shown to be a much stronger directing group than any other. Attempts to quantify the directing effect of a substituent by considering traditional metrics for steric size led to some confusion. A-values are a commonly used metric for steric size, representing the free energy difference of a mono-substituted cyclohexane ring in the more stable (equatorial) and less stable (axial) conformations.[153] The A-values

of the halogens (Table 8)[153] do not reflect the observed difference in regioselectivity. For esters, the A-values should roughly correspond to the A-values of the alkyl group of the alkoxide as the carboxylates remain constant. Thus, a comparison of methyl, ethyl, *n*-propyl, allyl, and the propargyl group could be made on the basis of the difference between terminal substituents (H for methyl ester, CH_3 for ethyl ester, ethyl for *n*-propyl ester, vinyl for allyl ester, and acetylene for propargyl ester). As with the halogen substituents, the A-values (Table 8) cannot be used to explain the directing effects of the ester substituents. Thus, other factors need to be considered to justify the observed regioselectivity trends.

Table 8: Metrics for the steric size of halogen and ester substituents.[153,154]



X =	A-value[153]	Charton value (ν)[154]	R =	A-value[153]	Charton value (ν)[154]
F	0.25-0.42	0.27	H	0	0
Cl	0.53-0.64	0.55	Me	1.7	0.52
Br	0.48-0.67	0.65	Et	1.8	0.56
I	0.47-0.61	0.78	vinyl	1.5	1.51
			alkynyl	0.4	-

Another metric to consider would be the Taft/Charton[154] steric parameter value (ν) for both halogens and ester alkyl groups. The Taft/Charton steric parameter is

calculated from the rates of acid-catalyzed hydrolysis of aliphatic esters.[154] Unlike the A-values, these follow a logical trend (Table 8), although the value for alkynyl is not available. While there is no clear linear relationship to explain the reversal of directing trends between the "apparent size" of the halogen versus that of the ester, at least some of the trends may be rationalized by using the internally consistent ν values for halogens and for the alkyl groups. For the esters it is clear that propargyl groups, regardless of apparent size, override the directing effects of halogens, as evidenced from the ratios of the two regioisomers obtained in all cases. Although it would be possible to speculate, at this time, and without additional information, the origin of the selectivity apparent for propargylic esters and the difference in observed yields for these substrates cannot be explained.

This study provides greater predictive power in determining the regioselectivity in the dihydroxylation of *ortho*-disubstituted arenes. This study also determined the steric limitations on the substrate scope of TDO with respect to *ortho*-halobenzoates, as all *tert*-butyl benzoates were shown not to be substrates for the enzyme. This results of this study were published in 2014.[155]

3.2.2 Dihydroxylation of *para*-disubstituted arenes

As part of an ongoing program in the synthesis of tetrodotoxin (**10**), a marine toxin first isolated in 1950,[156] a series of *para*-disubstitued arenes were investigated as substrates for TDO (Figure 34). In any approach to tetrodotoxin (**10**), a significant challenge would be the installation of the *syn*-carbon chains highlighted in Figure 34. An approach toward this compound was envisioned wherein the core structure could be accessed through the use of 1,4-disubstituted diene-diol metabolites (**9**). An ideal substrate (**8**) would include both carbon chains prior to enzymatic dihydroxylation, and a primary goal of the investigation of *para*-disubstituted arenes as substrates for TDO was to identify such a substrate.

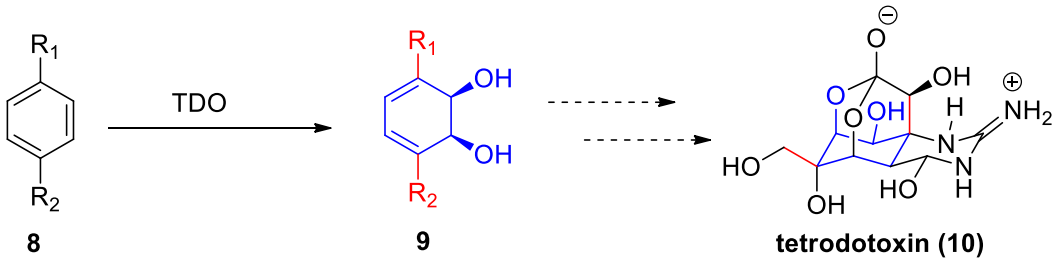


Figure 34: Potential synthesis of tetrodotoxin (**10**) using 1,4-disubstituted metabolites.

As discussed in Section 2.2.2.2, *para*-disubstituted arenes have previously been investigated as substrates for TDO by Boyd (Figure 17).[57] In the course of this study, Boyd quantified the ratio of enantiomeric metabolites produced, and found that the ratio was determined by the steric size of the substituents on the aromatic ring.[57] In designing a starting material for a potential enantioselective synthesis of tetrodotoxin (**10**), the enantioselectivity of the enzymatic dihydroxylation must be an important consideration.

Therefore, the goals of the investigation of *para*-disubstituted arenes as substrates for TDO must consider providing greater predictive power to Boyd's model,[57] in order to aid in the design of an appropriate starting material.

In order to investigate *para*-disubstituted arenes as substrates for TDO, small-scale (1 L) fermentation cultures of *E. coli* JM109 (pDTG601A)[54] were grown and fed with substrates according to reported procedures.[14(m),69] In order to determine if an aromatic compound was metabolized by TDO, the crude fermentation extract was analyzed by ^{1}H NMR. In cases where the metabolism of a given substrate was observed, large-scale (10 L) fermentations were performed,[14(m)] in order to provide an accurate measure of the fermentation yield and to provide sufficient material for characterization.

With the goal of identifying metabolites with carbon substituents at para-positions, compounds **278**-**283** (Figure 35) were tested as substrates for TDO. In each case, no conversion to the corresponding diene-diol metabolites was observed, and the aromatic starting material was recovered from the fermentation media.

Because of the lack of success in the metabolism of *p*-disubstituted substrates (**278**-**283**), halogenated substrates **284**-**288** (Figure 35) were investigated. These substrates may also be applied to the synthesis of tetrodotoxin, as the halogen substituent could be converted to the desired carbon substituent through coupling chemistry. Carboxylic acids **284**-**285** were shown not to be substrates for TDO, with the aromatic starting material being recovered from the fermentation media. Attempts to increase the solubility of these compounds, either by conversion to their corresponding sodium salts or by dissolution in dimethyl sulfoxide (DMSO) before feeding, produced identical results. Compounds **286** and **287** were shown not to be substrates for TDO, with the

starting material being recovered from the fermentation media. Compound **288** was shown to be consumed in the fermentation broth, although no conversion to the corresponding diene-diol metabolite was observed. Consumption of compound **288** by the fermentation culture presumably occurs through oxidation of the sulfur atoms, as has been reported.[157]

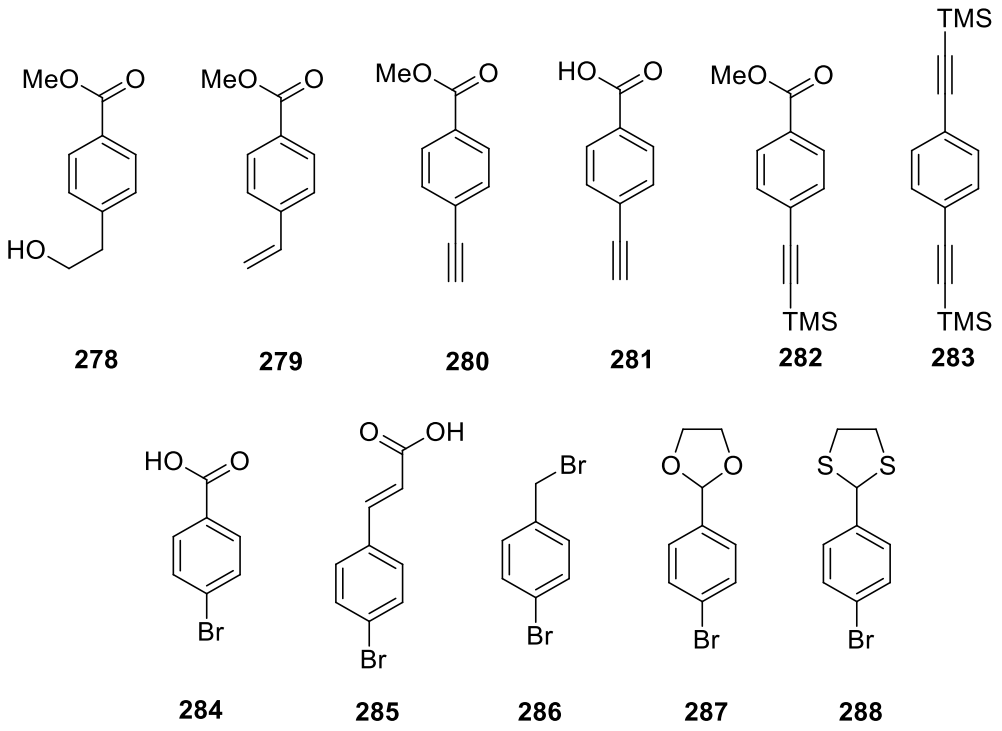


Figure 35: *para*-Disubstituted arenes shown not to be substrates for TDO.

Some success was achieved when halogenated substrates **289**-**292** were investigated, as trace amounts of the corresponding diene-diol metabolites **293**-**296** were detected in the crude fermentation extracts (Figure 36). Although the diene-diol metabolites **293**-**296** were detected by ^{1}H NMR analysis of the crude fermentation

extract, these compounds were not produced in quantities significant enough to facilitate isolation and characterization.

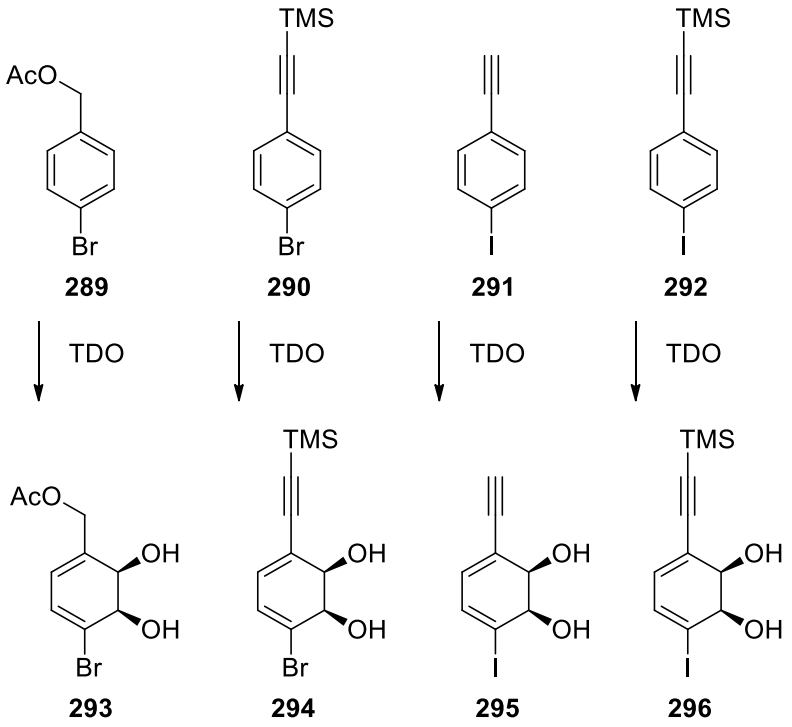


Figure 36: Metabolites produced in trace amounts from fermentation cultures. *Stereochemistry shown is based on analogy to related compounds.

In contrast, brominated arenes **297**-**300** were metabolized by TDO in reasonable yields from large-scale (10 L) fermentation cultures (Figure 37). Enantiomeric ratios (er) were determined through the use of chiral HPLC. Interestingly, the metabolism of both the alcohol and aldehyde compounds **297** and **298** afforded the same metabolite, triol **301**, in similar yields. The difference in enantiomeric ratios, however, indicates that the *in situ* aldehyde reduction occurs at least in part after the enzymatic dihydroxylation. Furthermore, the greater selectivity observed with aldehyde substrate **298** indicates that the aldehyde functionality has a more significant directing effect on the enzymatic

dihydroxylation when compared to the benzyl alcohol moiety. The use of the allyl substrate **299** led to a further increase in selectivity, with the allyl moiety directing the enzymatic dihydroxylation. The greatest level of selectivity was observed with alkynyl substrate **300**, with the alkyne substituent demonstrating the strongest directing effect of any substituent investigated in this study. This observation was in accordance with the particularly strong directing effect of propargyl ester substituents that was observed in the metabolism of *ortho*-halobenzoates by TDO (Section 3.2.1).

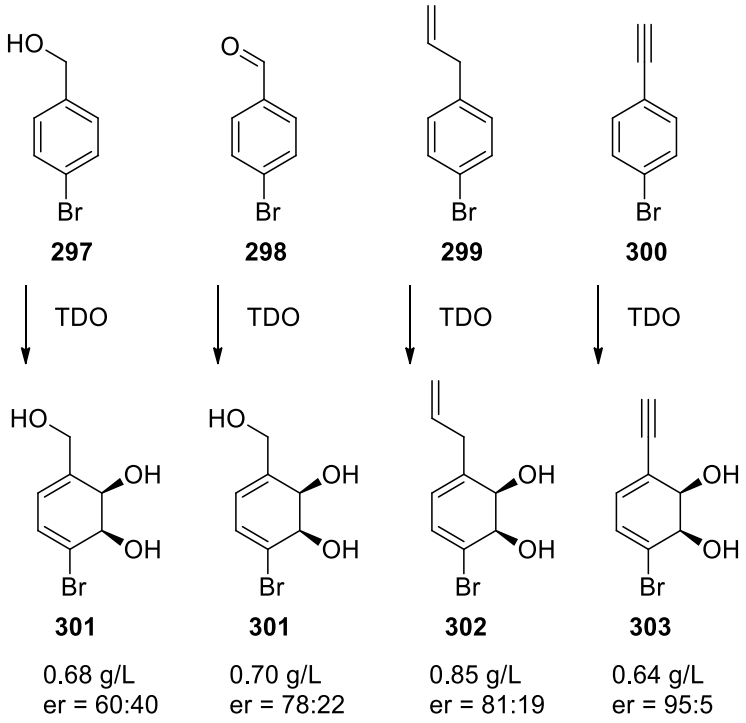
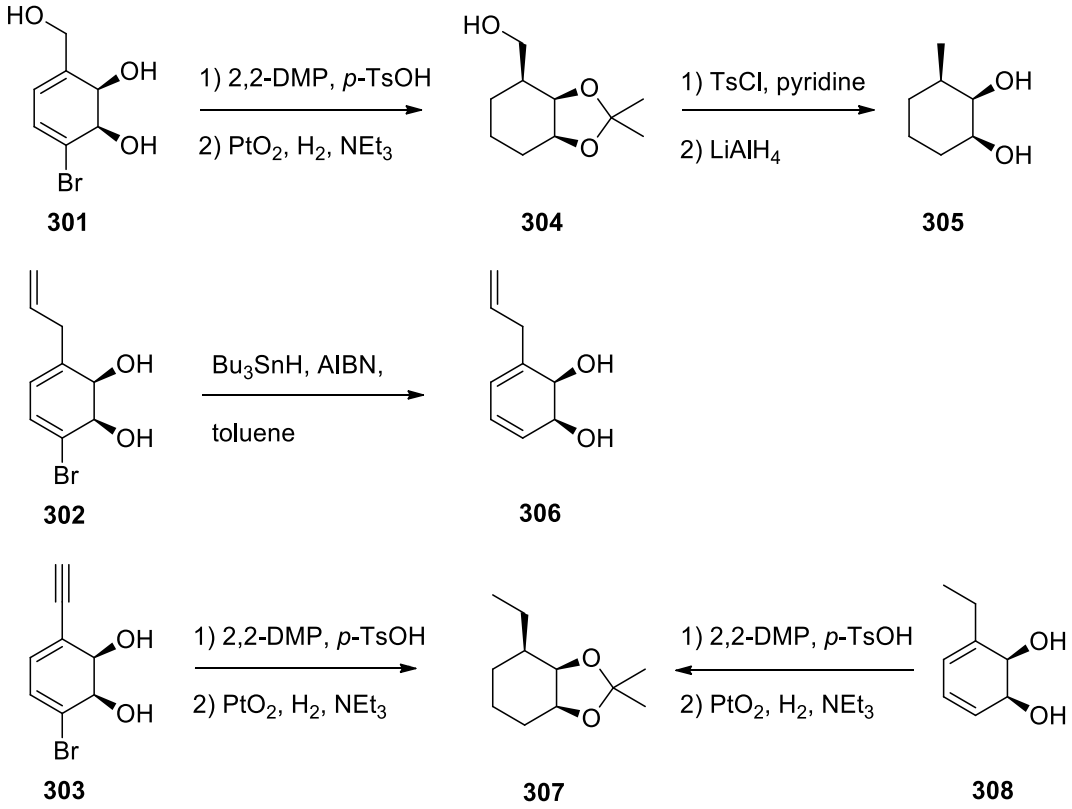


Figure 37: Metabolism of *para*-disubstituted arenes by TDO.

In order to confirm the absolute stereochemistry of diene-diol metabolites **301-303**, each compound was converted to a compound of known absolute stereochemistry (**305-307**) (Scheme 24). These derivatized compounds were matched to their previously reported counterparts by ^{1}H NMR and optical rotation. Metabolite **301** was protected as its corresponding acetonide, and hydrogenated with Adam's catalyst[158] to afford

cyclohexane **304**. Tosylation of **304** led to decomposition, presumably through elimination of the tosylate functionality and acid-catalyzed hydrolysis of the acetonide protecting group. Hydride reduction of the decomposition residue afforded the known diol **305**.[159] Allyl metabolite **302** was subjected to debromination under standard conditions to provide the known diol **306**.[160] Acetonide protection and hydrogenation of alkynyl metabolite **303** afforded the protected diol **307**, which was previously reported by Gibson in 1977.[161] This initial report however, did not provide any characterization data. Therefore, in order to match the derivatized compound, protected diol **307** was prepared from the known metabolite **308**,[160,162] and was matched to the sample prepared from metabolite **303**.



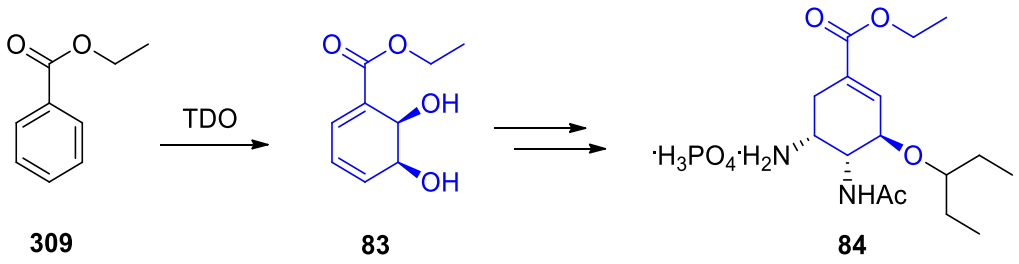
Scheme 24: Derivatization of metabolites **301**-**303** to confirm absolute stereochemistry.

Although this study did identify three new *para*-disubstituted metabolites (**301-303**), one of which was produced in a reasonably high enantiomeric ratio (**303**), none of these compounds have yet been applied in the synthesis of tetrodotoxin (**10**). For more information on this topic, see Section 3.4. This study did provide valuable information as to the limits of the substrate scope of TDO with respect to *para*-disubstituted arenes. This information will aid in planning of future synthetic sequences from other substrates. Furthermore, analysis of the enantiomeric ratios of metabolites **301**-**303** produced by TDO (Figure 38) provides information on the relative directing effect of the substituents on the aromatic precursors. From this information, the relative directing effect of these substituents can be described as follows: alkyne > allyl > aldehyde > alcohol > bromine. This observation is in accordance with the particularly strong directing effects exhibited by propargyl ester substituents in the investigation of *ortho*-halobenzoates.[155] The results of this study were published in 2013.[163]

3.3 Carbonylation of halo *cis*-cyclohexadiene diols to the corresponding carboxylates

3.3.1 Development and substrate scope of carbonylation methodology

In 2010, Hudlicky published an efficient synthesis of the potent anti-viral compound oseltamivir (**84**), which used the enzymatic metabolite of ethyl benzoate, diol **83**, as a starting material (Scheme 25).[82] The success of this work led to a collaboration with Professor Machara (Charles University, Prague) with the goal of applying Hudlicky's methodology to produce analogs of oseltamivir (**84**).[164] Subjecting these analogs to biological testing would provide information on the structure-activity relationship of these compounds, and potentially identify compounds that are more active than oseltamivir.

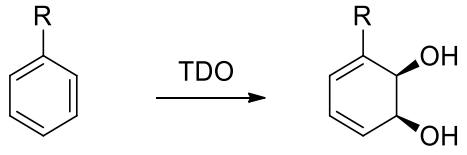


Scheme 25: Synthesis of oseltamivir (**84**) from diol **83** by Hudlicky.[82]

A major obstacle in the production of a library of oseltamivir analogs is the efficient production of metabolites of type **83** in large quantities. As is evident from Table 9, the metabolites of benzoates are produced by TDO in relatively low titres,[66] particularly when compared to the titres obtained with halogenated aromatics.[152] In order to pursue the production of oseltamivir analogs, as well as other syntheses that require

metabolites of type **83** (see section 3.4.2), a means of producing these compounds in greater yields was required.

Table 9: Yields of the enzymatic dihydroxylation of halogenated aromatics and benzoates.[66,152]

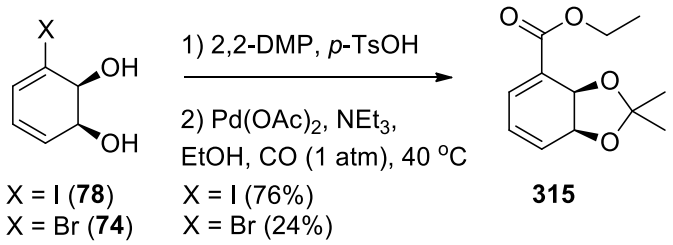


R =	Yield (g / L)	Reference
I (**78**)	10	[152]
Br (**74**)	20	[152]
Cl (**85**)	12	[152]
CO_2Me (**310**)	1.5	[66]
CO_2Et (**83**)	1.0	[66]
CO_2*n*-Pr (**311**)	0.07	[66]
CO_2*i*-Pr (**312**)	0.05	[66]
CO_2*t*-Bu (**313**)	-	[66]
CO_2Bn (**314**)	-	[152]

Palladium-catalyzed carbonylation of halogenated metabolites such as diols **74** and **78** was investigated as a means for the production of esters of type **83** (Scheme 26). Some precedent for this process was provided by Boyd, who prepared a number of compounds through the cross-coupling of metabolites of this type, in some cases with the diene functionality being partially saturated.[165] Furthermore, Banwell had successfully

performed the sp^2-sp^3 coupling of the vinyl iodide moiety of metabolite **78** at elevated temperatures.[166]

For the preliminary investigation of the palladium-catalyzed carbonylation methodology, iodo-metabolite **78** was chosen as the starting material because of the increased reactivity of the vinyl iodide functionality relative to other halogens.[107,109] The diol moiety of metabolite **78** was protected as its corresponding acetonide prior to being subjected to the carbonylation conditions. This protection step was performed because of the increased stability of acetonide-protected derivatives relative to their corresponding free diols. Carbonylation conditions (Scheme 26) were adapted from initial reports by Heck,[107] and from reports of similar reactions performed by Boyd.[165] These conditions were chosen based upon their past success, and because of the relatively low cost of the palladium (II) source ($Pd(OAc)_2$) relative to other commercial palladium catalysts.

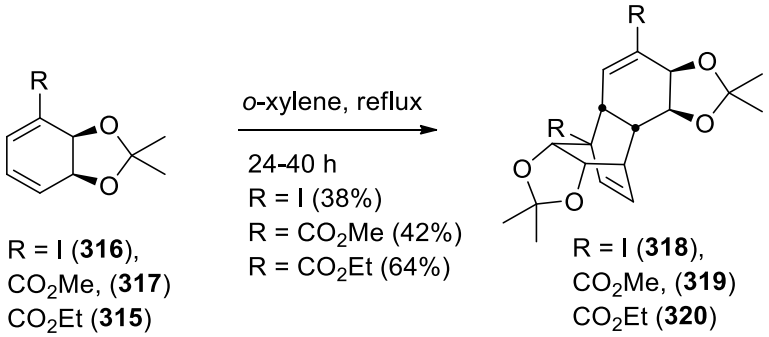


Scheme 26: Initial study of the palladium-catalyzed carbonylation of halogenated metabolites.

To our delight, the initial reactions were effective, with acetonide **315** being produced in good yields from the acetonide derived from metabolite **78** (Scheme 26). A better substrate was metabolite **74**, as it was produced in high titres from enzymatic dihydroxylation (Table 9).[152] Unfortunately, carbonylation reactions performed with this

substrate afforded significantly lower yields (Scheme 26). Thus, iodo-metabolite **78** was established as the standard substrate for this methodology.

Acetonide-protected metabolites of type **315** are well known to undergo stereoselective Diels-Alder type dimerization even at low temperatures.[167] In order to monitor this unwanted side-reaction during the study of the carbonylation methodology, dimers **318**-**320** were prepared from their corresponding monomers by refluxing a solution of these compounds in xylene (Scheme 27).

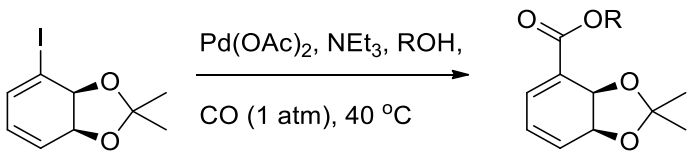


Scheme 27: Synthesis of dimers **318**-**320**.

With the success of the initial study of the palladium-catalyzed carbonylation reaction (Scheme 26), the substrate scope was subsequently expanded (Table 10). This was accomplished by performing the reaction in different alcoholic solvents, or in the case of compounds **323**-**326**, performing the reaction in a solution of the desired alcohol and THF (1:9). The reaction was shown to be quite versatile, as good yields were obtained for many substrates. Performing the reaction in the presence of allyl alcohol, propargyl alcohol or *t*-butyl alcohol led to increased reaction times, and correspondingly

lower yields. Despite initial concerns, little to no dimerization of either the acetonide starting materials or the ester products was observed in most cases.

Table 10: Palladium-catalyzed carbonylation of acetonide **314** to corresponding esters.

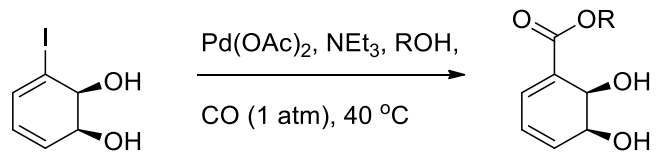


R =	Yield
Me (**317**)	68%
Et (**315**)	73 %
n-Pr (**321**)	68 %
i-Pr (**322**)	76 %
t-Bu (**323**)	10 %
allyl (**324**)	27 %
propargyl (**325**)	20 %
benzyl (**326**)	70 %

Having established the applicability of this methodology to acetonide-protected metabolites of type **315**, this study was expanded to include the carbonylation of free diol **78** (Table 11). Carbonylation reactions were similarly successful using free diol **78** as a substrate, as comparable yields to those obtained with acetonide **315** were observed in most cases. As was previously observed, reactions performed in the presence of allyl

alcohol, propargyl alcohol or *t*-butanol resulted in longer reaction times and correspondingly lower yields.

Table 11: Palladium-catalyzed carbonylation of diol **78** to corresponding esters.

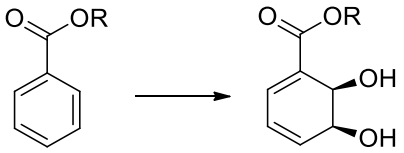
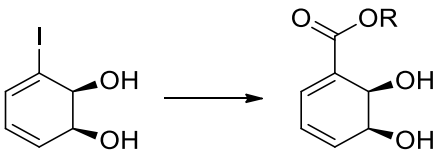


R =	Yield
Me (**310**)	68 %
Et (**83**)	72 %
n-Pr (**311**)	62 %
i-Pr (**312**)	72 %
t-Bu (**313**)	17 %
allyl (**327**)	6 %
propargyl (**328**)	36 %
benzyl (**314**)	10 %

In an attempt to quantify the efficiency of the palladium-catalyzed carbonylation methodology, and compare the amounts of esters **83**, **310**-**315**, **327**, **328** available through the two methods, quantities produced are shown in Table 12. The first column shows the total amount of each ester available through direct enzymatic dihydroxylation, when one large-scale (10 L) fermentation is performed. The second column shows the total amount of each ester available when one large-scale (10 L) fermentation is performed using

iodobenzene as the substrate, and the isolated metabolite **78** (100 g)[152] is subjected to carbonylation conditions. Although this metric does not take into account factors such as man-hours required to perform the carbonylation chemistry and the cost of the palladium catalyst, the benefits of the carbonylation chemistry are clear from Table 12. For example, in the production of ester **83**, the desired substrate for the synthesis of oseltamivir analogues, over five times more material is available through the carbonylation methodology compared to the direct dihydroxylation of ethyl benzoate. Performing large-scale biotransformations is considered to be the primary factor in determining the efficiency of these processes, due to the time and man hours involved. For this reason, it can be said that the carbonylation process is significantly more efficient as a means of producing ester **83**.

Table 12: Comparison of yields through direct dihydroxylation and through carbonylation.

		
R =	Yield by direct fermentation (g)[66]	Yield by carbonylation (g)*
Me (**310**)	15	49
Et (**83**)	10	56
n-Pr (**311**)	0.7	52
i-Pr (**312**)	0.5	60
t-Bu (**313**)	-	15
allyl (**327**)	6	6
propargyl (**328**)	10	29
benzyl (**314**)	-	10

* [a]Hypothetical yield for comparison. Biotransformation of iodobenzene affords up to 100 g of the metabolite **78**. This yield was multiplied by the actual yield of the subsequent carbonylation step to reflect the relative amount available through this method. (Ex. (100 g (biotransformation yield) / 238.02 g/mol) x 0.72 (carbonylation yield) x 184.19 g/mol = 56 g)

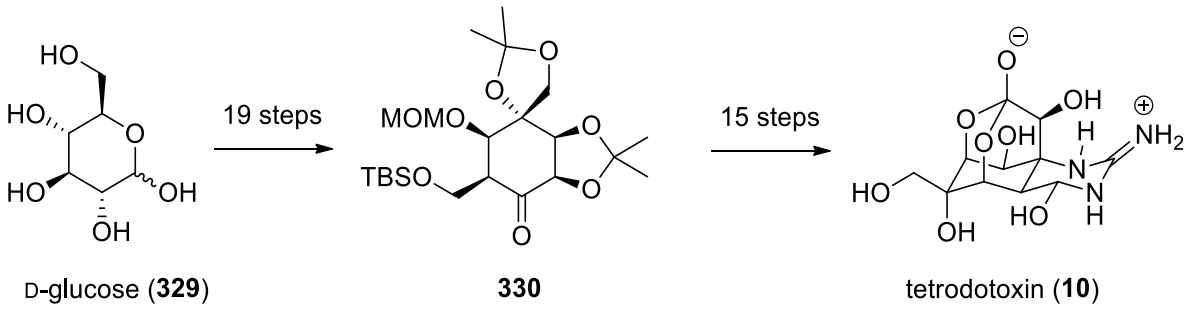
In examining table 12, a second benefit of the palladium-catalyzed carbonylation methodology becomes evident, namely the potential to access ester compounds such as **313** and **314**, which are unavailable through direct enzymatic dihydroxylation.[66,152] Previous studies have demonstrated that *t*-butyl benzoate and benzyl benzoate are not substrates for TDO, presumably due to the steric size of the ester substituents.[66,152] By

applying the carbonylation methodology, ester metabolites with sterically large substituents, including but not limited to **313** and **314**, can be accessed.

With the evident increase in efficiency afforded by the carbonylation methodology, this chemistry was applied in the production of oseltamivir analogues from ester **83** by Professor Machara.[164] Furthermore, this chemistry was applied as part of the continued interest in developing a synthetic route towards tetrodotoxin (see sections 3.2.2, 3.3.2). This work was published in 2014.[152]

3.3.2 Approach towards the synthesis of tetrodotoxin

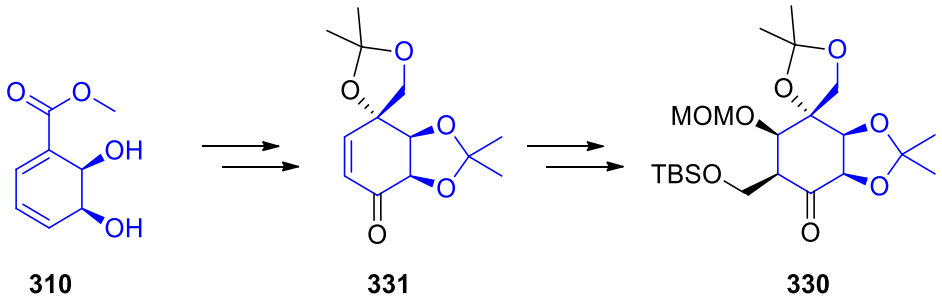
Because the study described in section 3.2.2 failed to identify a substrate for TDO with carbon substituents at the *para* positions, or a metabolite that was produced with complete enantioselectivity, an alternate route towards the synthesis of tetrodotoxin was envisioned. This new synthetic plan was inspired by a report by Sato, who prepared tetrodotoxin (**10**) from D-glucose (**329**) in 2008 (Scheme 28).[168] Sato's synthesis used ketone **330**, which was synthesized in 19 steps from D-glucose (**329**), as a key intermediate.



D-glucose (**329**) **330** tetrodotoxin (**10**)

Scheme 28: Synthesis of tetrodotoxin (**10**) from D-glucose (**329**) by Sato.[166]

The goal of this synthetic study was to develop an alternate route to ketone **330**, which would constitute a formal synthesis of tetrodotoxin (**10**). The synthetic strategy that was applied was based on the production of enone **331**, which could be readily prepared from enzymatic metabolite **310** (Scheme 29). Enone **331** could then be elaborated to ketone **330**, completing the synthesis of this compound in significantly less than 19 steps. If ketone **330** could be produced from metabolite **310** in less than 19 steps, this would constitute a major advancement in tetrodotoxin synthesis.

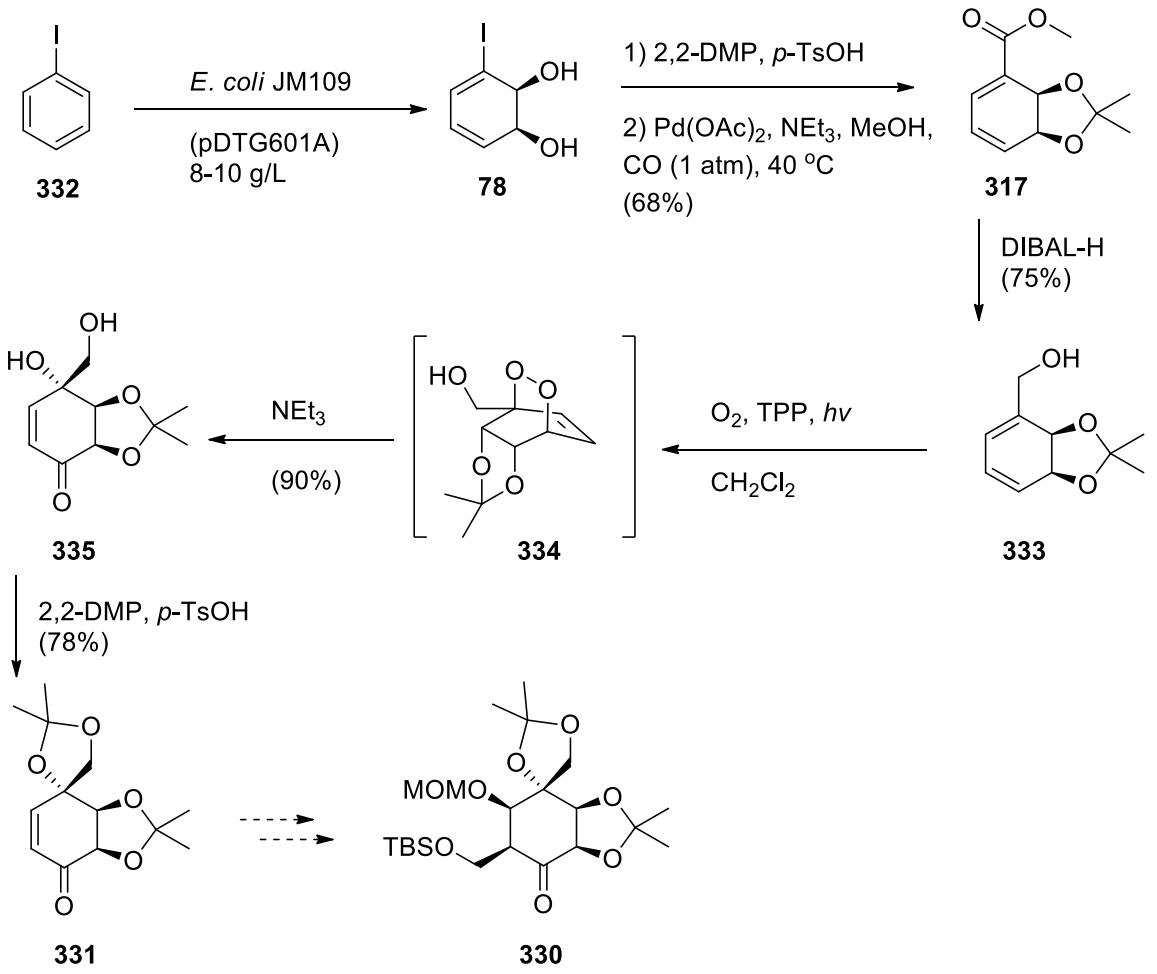


310 **331** **330**

Scheme 29: Synthetic strategy for the production of ketone **330**.

Having developed a methodology for the palladium-catalyzed carbonylation of halogenated enzymatic metabolites (Section 3.3.1), the acetonide-protected ester **317** was readily prepared from metabolite **78**. This methodology was employed because of the relatively low titres obtained in the enzymatic dihydroxylation of methyl benzoate, when compared to those obtained with iodobenzene (Table 8, Section 3.3.1). Ester **317** was subsequently reduced with diisobutylaluminum hydride (DIBAL-H) to afford alcohol **333**. This compound was shown to dimerize rapidly upon concentration to dryness, and was therefore immediately carried on to the subsequent step without further purification. The diene functionality of alcohol **333** was then subjected to a facially-selective [4+2] cycloaddition with singlet oxygen,[169] as has been described in Lewis' synthesis of (+)-3-*O*-debenzoylzeylenone (**195**) (Section 2.4.3).[100] This stable endoperoxide (**334**) was then treated with base *in situ* to effect the desired Kornblum-DeLaMare rearrangement.[170] This was accomplished either by addition of the base directly to the reaction mixture upon completion of the [4+2] cycloaddition, or by chromatography of endoperoxide **334** on silica, which was deactivated with 5% triethylamine. This tandem [4+2] cycloaddition/Kornblum-DeLaMare rearrangement process proceded smoothly, affording

diol **335** in 90% yield. Diol **335** was then protected as its corresponding bis-acetonide to provide enone **331**.



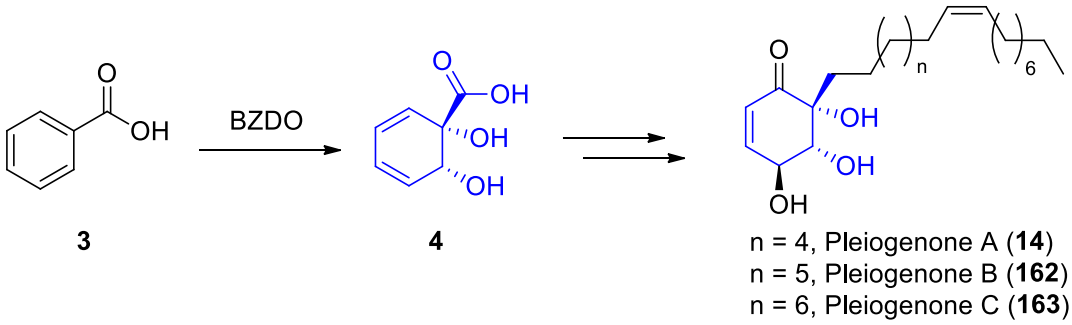
Scheme 30: Synthesis of enone **331** from metabolite **78**.

Having developed a highly efficient route to enone **331** (6 steps from **78**, 36% overall yield, 2 chromatography steps), we produced multi-gram quantities of this key intermediate. Although the ultimate goal of this work has yet to be accomplished, studies are ongoing in the Hudlicky group to convert enone **331** to ketone **330**, which would constitute a formal synthesis of tetrodotoxin (**10**).

3.4 Synthesis of pleiogenone A

In 2015, Kingston reported the isolation of pleiogenones A-C (**14**, **162**, **163**) from the bark of *Pleiogynium timorense* (Figure 31).[17] In this initial report, pleiogenones A-C were tested for their anti-proliferative activity against the A2780 ovarian cancer cell line and demonstrated IC_{50} values of 0.8, 0.7 and 0.8 μM, respectively.[17] Based upon the promising biological activity of these compounds, it was determined that they would be valuable synthetic targets. Such a synthesis would serve as a proof of the structure and absolute stereochemistry of these compounds, and would permit their production in quantities large enough to facilitate further biological testing. Furthermore, the development of a modular synthesis would allow for the synthesis of all pleiogenone congeners, as well as analogs of these compounds for future structure-activity relationship studies.

Inspired by the work of Lewis in the synthesis of 3-*O*-debenzoylzeylenone (**195**, Scheme 20, Section 2.4.3),[100] it was clear that the polyhydroxylated cyclohexenone core of the pleiogenones could be accessed from *ipso*-diol **4** (Scheme 31). The key strategy in the construction of the cyclohexenone core would be the [4+2] cycloaddition of singlet oxygen to the diene moiety, as was employed by Lewis,[100] and in our previously discussed approach towards tetrodotoxin (**10**, Scheme 30, Section 3.3.2).



Scheme 31: Strategy for the synthetic approach towards pleiogenones A-C (**14**, **162**, **163**).

The primary synthetic challenge of any synthetic approach towards pleiogenones A-C (**14**, **162**, **163**) is the construction of the alkenyl side-chain, in this case from the carboxylate moiety of *ipso*-diol **4**. Three potential strategies were envisioned for the construction of the alkenyl side-chain, each of which employed alcohol **336** as a key intermediate (Figure 38). The first strategy involved the Negishi-type sp^3-sp^3 coupling[171] of an activated alcohol (**337**) derived from **336**, with an alkyl-zinc reagent as has been described.[171(b)] The second strategy involved the Grignard addition[172] to an aldehyde derived from **336**, and subsequent deoxygenation of the resulting alcohol (**338**). The final strategy that was envisioned was based on a Wittig olefination[173] of aldehyde **340**, which would require the hydrogenation of the alkene moiety (**341**) at some point in the synthesis.

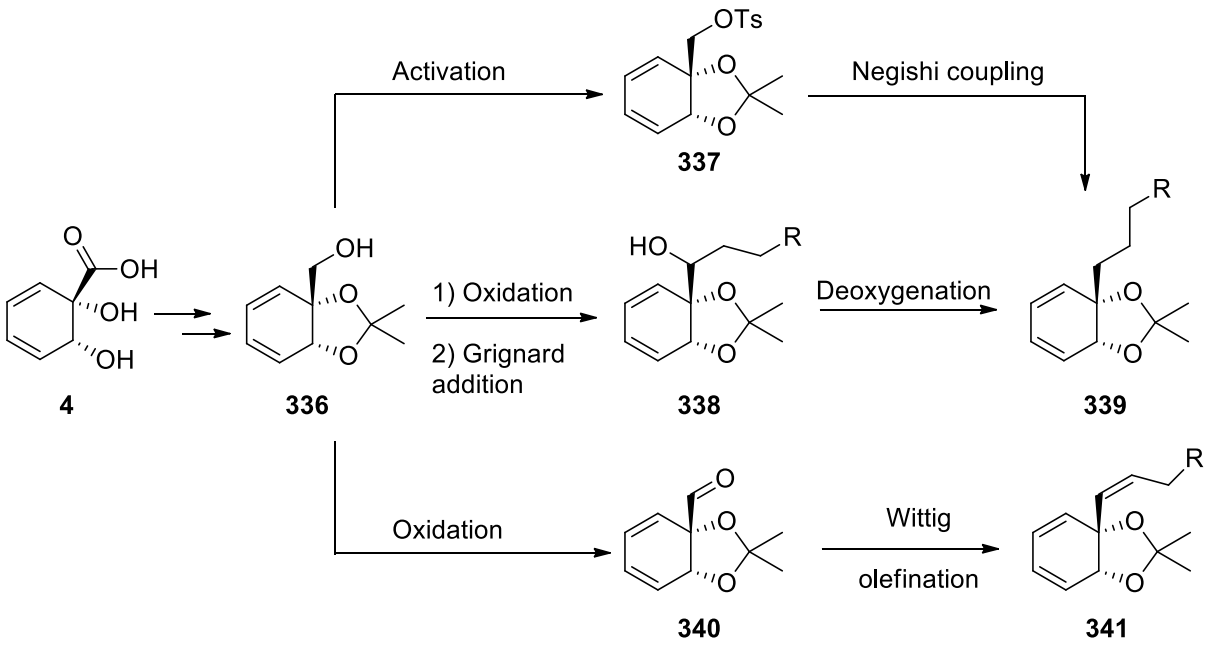
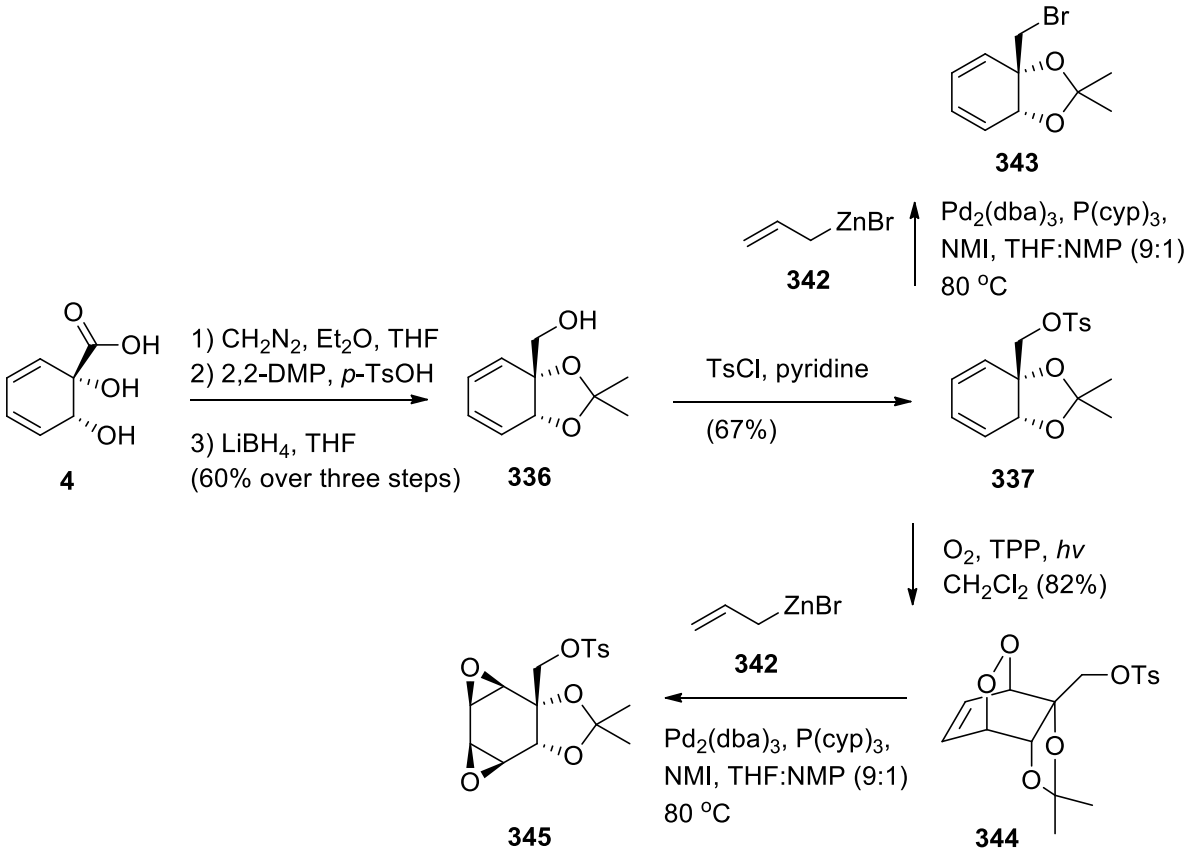


Figure 38: Potential strategies for the construction of the pleiogenone alkenyl side-chain.

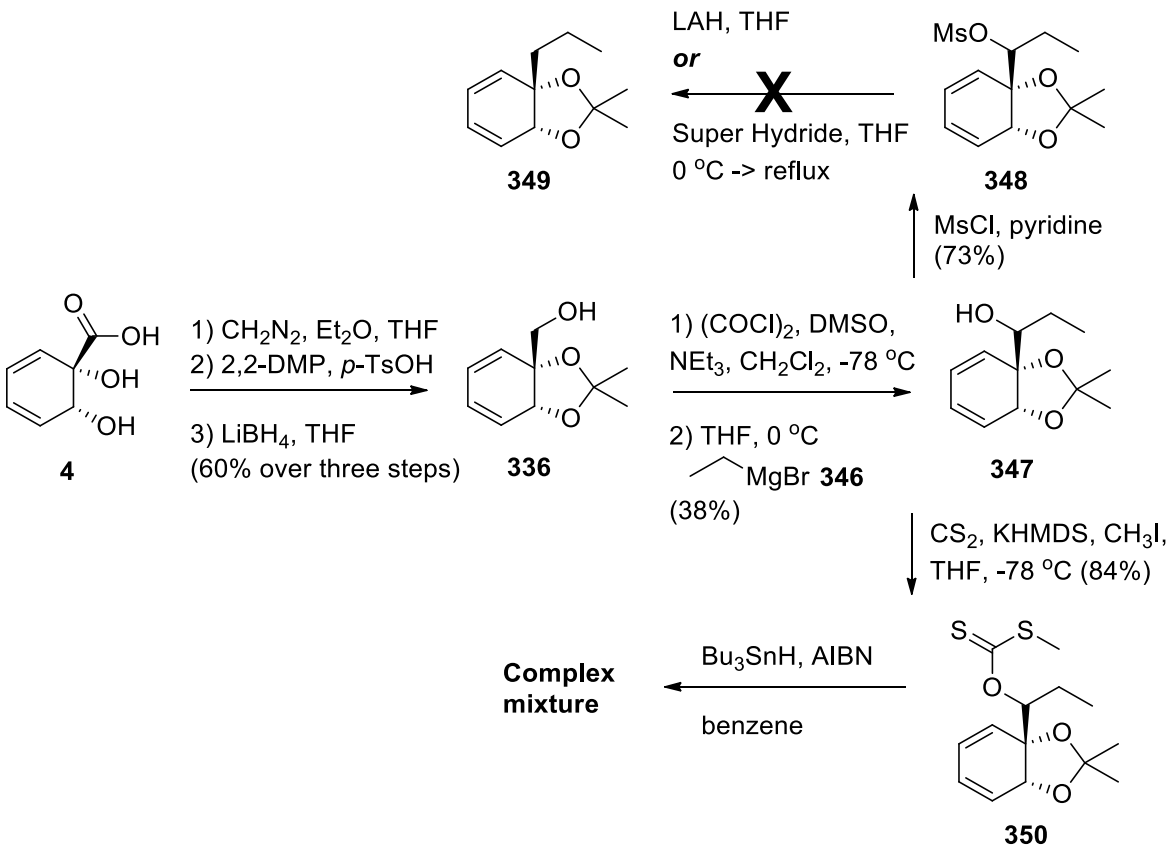
The first strategy for the construction of the pleiogenone alkenyl side-chain to be investigated was the Negishi-type coupling strategy. This strategy was considered the most likely to lead to an efficient synthesis, as it would not require further manipulation of the alkenyl side-chain after the coupling step. In order to investigate this route, model studies were pursued through the key intermediate tosylate **337**. Tosylate **337** was synthesized from *ipso*-diol **4**, through a series of transformations shown in Scheme 32. The conditions for the Negishi-type coupling used in this model study were taken from recent reports of the sp^3-sp^3 coupling of alkyl tosylates and alkyl zinc reagents.[171(b)] To test the applicability of these conditions to the coupling of tosylate **337**, alkyl zinc reagent **342** was prepared and combined with tosylate **337** under the reported conditions. Unfortunately, none of the coupled product was observed under these conditions, with only tosylate **337** and a small amount (<10%) of bromide **343** being recovered after

prolonged reaction times. This result could have been anticipated from previous reports, which have indicated that the relevant carbon of tosylate **337** is particularly sterically hindered.[174] In an attempt to reduce the steric crowding of the relevant position, tosylate **337** was converted to endoperoxide **344**, which was treated under identical conditions in the Negishi-type coupling. Unfortunately, heating endoperoxide **344** under conditions for the Negishi-type coupling resulted in a rearrangement to bis-epoxide **345**. This result could also have been anticipated, as the rearrangement of cyclic alkenyl endoperoxides to their corresponding bis-epoxides has been reported.[175]



Scheme 32: Model studies on Negishi-type coupling route towards pleiogenones A-C.

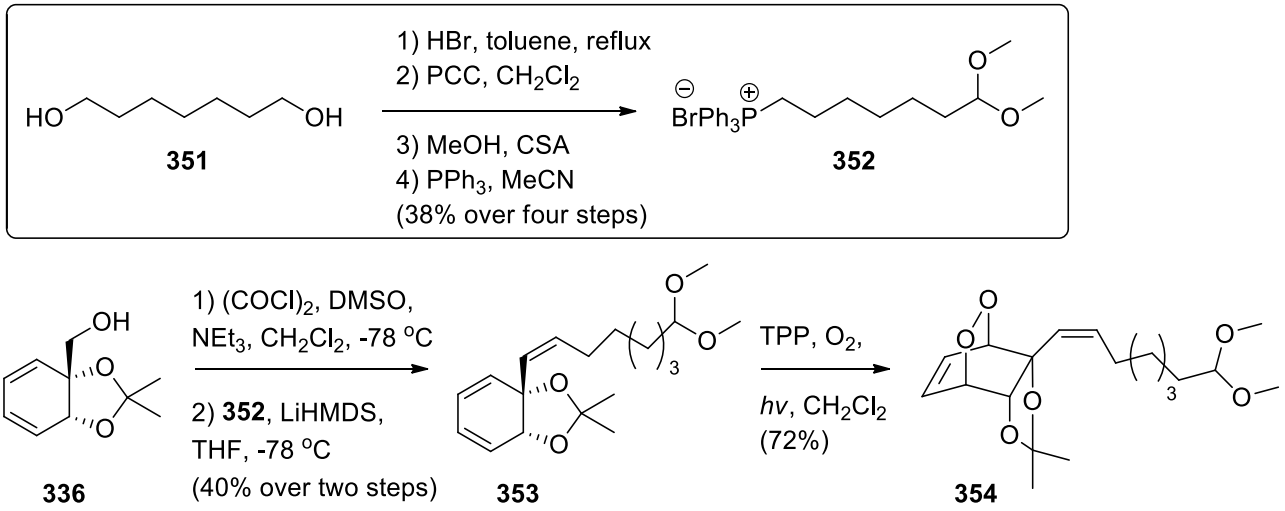
The second strategy for the construction of the pleiogenone alkenyl side-chain to be investigated was the Grignard addition / deoxygenation approach. To this end, alcohol **336** was oxidized to the corresponding aldehyde, which was subjected to a Grignard addition with the preformed Grignard reagent **346** (Scheme 33). The alcohol moiety of the resultant compound **347** was then activated as its corresponding mesylate **348**, in order to facilitate the hydride displacement of this moiety. Unfortunately, the treatment of mesylate **348** with various hydride sources resulted in no reaction from 0 ^{o}C to 23 ^{o}C, even after prolonged reaction times. Heating mesylate **348** to reflux with super hydride ($LiEt_3BH$) led to no reaction, while heating to reflux with lithium aluminum hydride (LAH) led to decomposition. This can be attributed to the steric congestion associated with the neopentyl carbon, as has been reported.[174] In order to investigate a potential radical deoxygenation, compound **347** was converted to the corresponding xanthate **350**, which was subjected to conditions for the Barton-McCombie deoxygenation,[176] producing a complex intractable mixture.



Scheme 33: Model studies on Grignard/deoxygenation route towards pleiogenones A-C.

Given the lack of success in the Negishi coupling and the Grignard/deoxygention model studies, the Wittig olefination/hydrogenation approach was the most likely to lead to an efficient synthesis of pleiogenones A-C, and pleiogenone A (**14**) was chosen as the synthetic target. Anticipating some issues involved in the selective hydrogenation of Wittig olefination products, it was determined that the alkenyl side-chain should be constructed in a modular fasion. This strategy would also lead to a versatile process that could be adapted to produce compounds with alkenyl side-chains of various lengths and substitution patterns. To pursue this strategy, Wittig reagent **352** was synthesized from 1,2-heptanediol (**353**), according to reported protocols (Scheme 34).[177] Although reagent

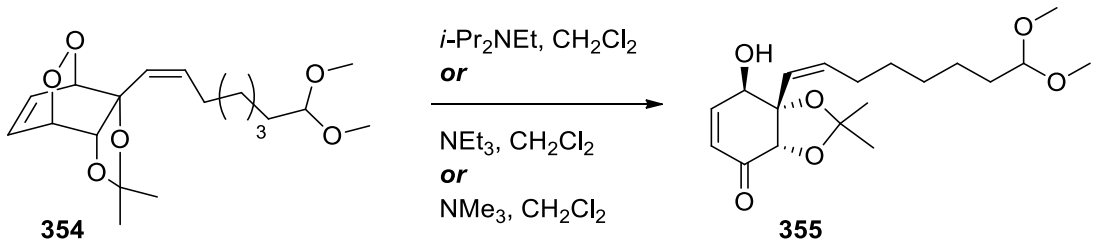
352 proved difficult to purify, its identity was confirmed through high-resolution mass spectrometry (HRMS) of the phosphonium cation (HRMS (ESI) calcd. for $C_{27}H_{34}O_2P^+$: 421.2296, found: 421.2295). Wittig reagent **352** was coupled with the aldehyde derived from alcohol **336**, affording triene **353** in 40% yield over two steps (Scheme 34). Further observation identified the instability of the aldehyde intermediate as the likely cause of this relatively low yield. Triene **353** was then subjected to the key [4+2] cycloaddition with singlet oxygen to afford endoperoxide **354**, which was shown to be bench-stable for short periods of time and was isolated to facilitate partial characterization (NMR, optical rotation).



Scheme 34: Synthesis of endoperoxide **354** from alcohol **336**.

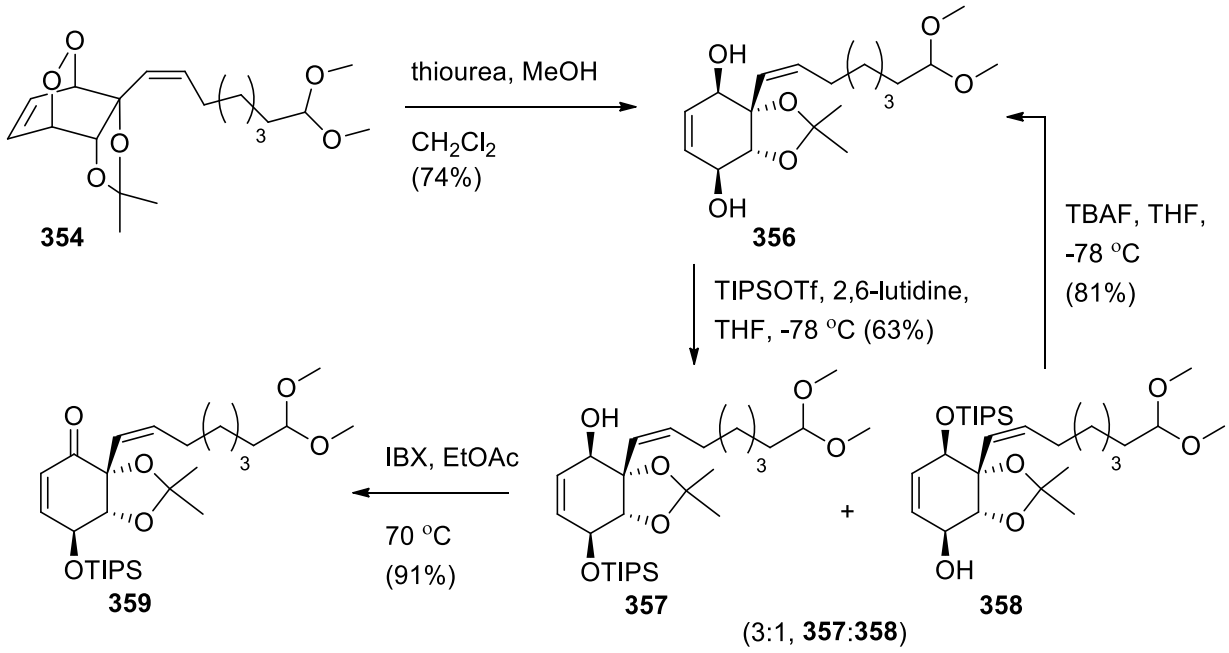
With endoperoxide **354** in hand, the Kornblum-DeLaMare rearrangement[170] of this compound was performed with bases of various steric bulk (Scheme 35). Unfortunately, these reactions led to the exclusive production of enone **355**, possessing the incorrect regiochemistry for the synthesis of pleiogenone A. This occurs because of

the exclusive deprotonation of the less sterically hindered site of endoperoxide **354**. These results mirror those reported by Lewis in the synthesis of 3-*O*-debenzoylzeylenone (**195**).[100]



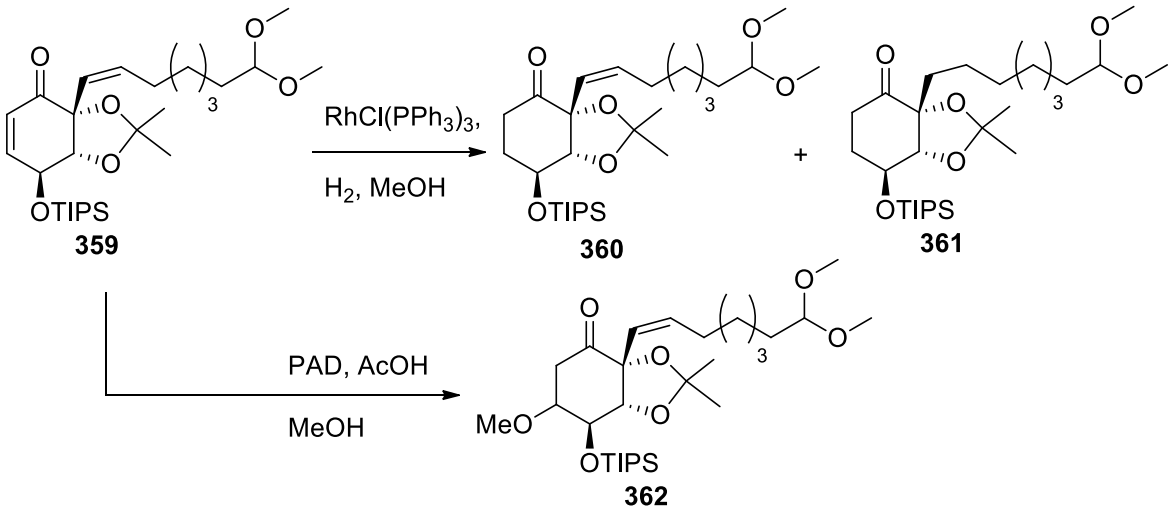
Scheme 35: Kornblum-DeLaMare rearrangement of endoperoxide **354**.

As the Kornblum-DeLaMare rearrangement was shown to proceed exclusively with the incorrect regiochemistry, an alternate strategy was employed wherein the endoperoxide moiety of **354** was reduced with thiourea to afford diol **356** (Scheme 36). Although endo-peroxide **354** was initially isolated for characterization, the cycloaddition and reduction steps in subsequent experiments were combined into a single operation without the need of isolation or purification. Taking advantage of the steric hindrance associated with diol **356**, the selective protection of the less hindered alcohol was accomplished according to a procedure reported by Banwell.[178] Although this protection step was not entirely selective, the undersired isomer (**358**) was recycled through deprotection to diol **356** and subsequent reprotection. Alcohol **357** was then oxidized with 2-iodoxybenzoic acid (IBX), producing enone **359** (Scheme 36).



Scheme 36: Synthesis of enone **358** from endoperoxide **354**.

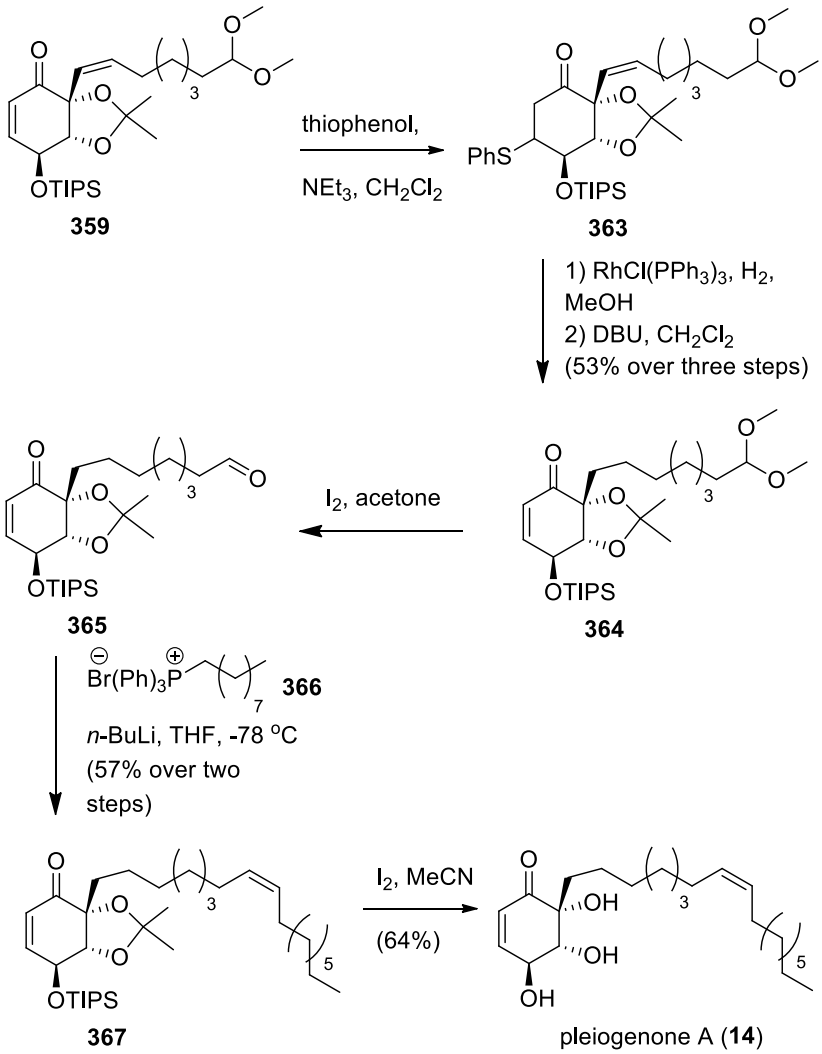
Enone **359** was considered the desired substrate for selective hydrogenation, based on previous reports of the selective hydrogenation of exocyclic olefins in the presense of enone functionalities with Wilkinson's catalyst ($RhCl(PPh_3)_3$).[179] Despite these reports, attempts at the selective hydrogenation of enone **359** resulted in a mixture of hydrogenated ketones **360** and **361** (Scheme 37). Attempts at diimide reduction in nucleophilic solvents resulted in Michael-type addition of solvent to the enone moiety (**362**), while experiments in non-nucleophilic solvents resulted in no reaction.



Scheme 37: Attempted selective hydrogenation of enone **359**.

To overcome the issues associated with the selective hydrogenation of the exocyclic olefin, the enone moiety was protected prior to hydrogenation. This task was accomplished through the Michael-type addition of thiophenol to enone **359** (Scheme 38), as has previously been reported for similar compounds.[180] Thiophenol adduct **363** was then subjected to hydrogenation with Wilkinson's catalyst. Experiments with catalytic amounts of Wilkinson's catalyst (10-20 mol %) invariably led to incomplete hydrogenation, however the use of one equivalent resulted in full hydrogenation. The deprotection of the thiophenol adduct with 1,8-diazabicycloundec-7-ene (DBU) allowed for the regeneration of the enone,[180] affording enone **364** (Scheme 38). The remaining challenge in the synthesis of pleiogenone A (**14**) from enone **364** was the completion of the alkenyl side-chain. Reports on the deprotection of acetal/ketal functionalities indicated that dimethyl acetal groups could be selectively deprotected in the presence of an acetonide using a catalytic amount of iodine in acetone.[181] This selective deprotection

of the dimethyl acetal functionality of enone **364** was successful, with complete removal of this functionality observed within five minutes. The resulting aldehyde (**365**), without further purification, was immediately subjected to a Wittig reaction with reagent **366**. This Wittig reaction smoothly afforded olefin **367**, with all of the functionality of pleiogenone A (**14**) in place. The Z-isomer (**367**) was the sole isomer obtained upon purification of the crude reaction mixture. A final deprotetion of the acetonide and silyl protecting groups was performed with iodine in acetonitrile (MeCN),[182] affording pleiogenone A (**14**) in a total of 16 steps (14 operations) and in 1.4% overall yield.



Scheme 38: Completion of the synthesis of pleiogenone A (**14**).

A sample of the natural product was kindly provided by Professor Kingston. The ^{1}H NMR spectra of the natural sample and the synthetic sample were shown to be a match (see section 6 for spectral comparison). The synthetic sample of pleiogenone A (**14**) was also matched with the natural sample by comparison of their optical rotation values. The match between the natural and synthetic samples also served to confirm the structure and absolute stereochemistry of the natural product as proposed by Professor Kingston.[17] The results of this study were published in 2016.[183]

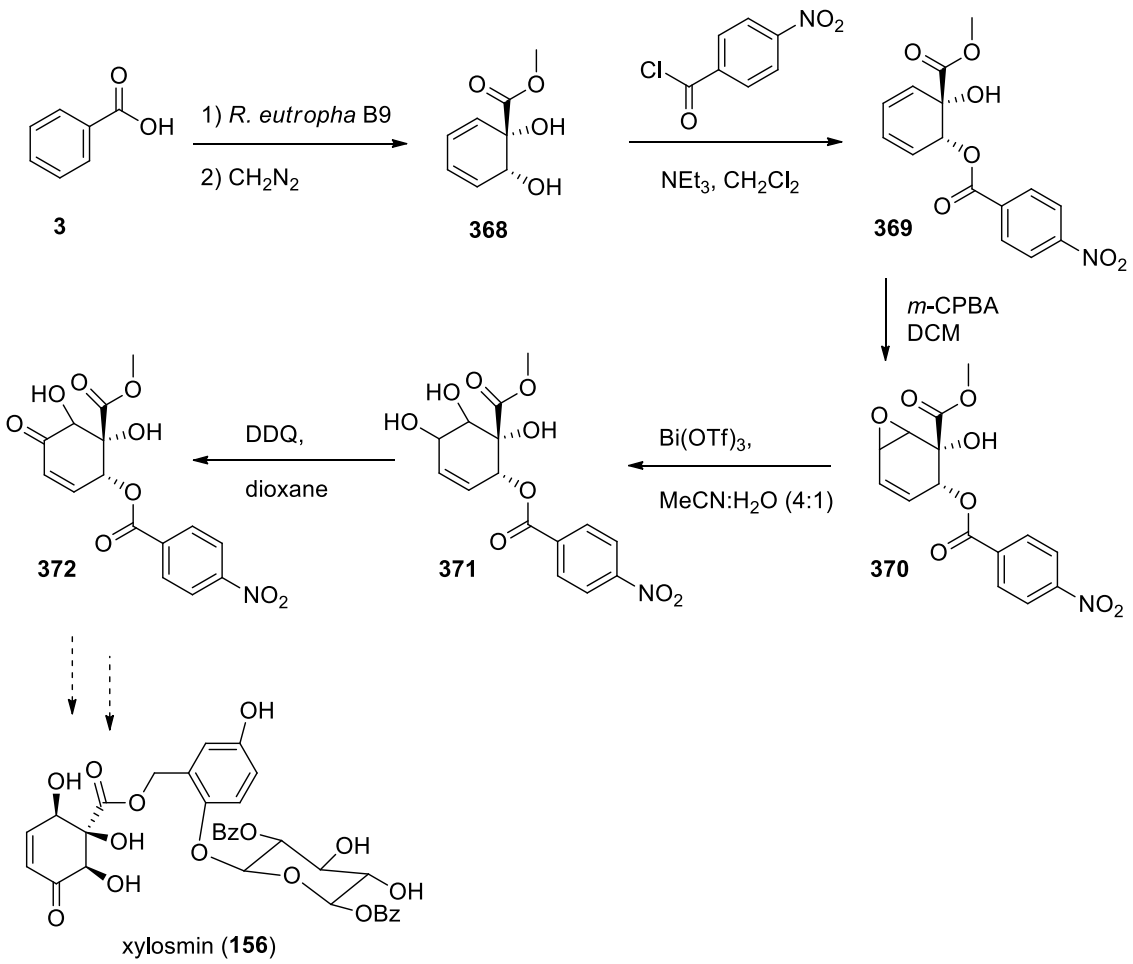
4. Conclusions and Future Work

The enzymatic metabolites of substituted aromatics produced by dioxygenase enzymes have proven to be valuable enantioenriched building blocks for the total synthesis of complex targets. It is hoped that the body of work presented here has provided a greater understanding of the substrate scope and selectivity of the important dioxygenase enzyme toluene dioxygenase (TDO), by investigating *ortho*- and *para*-disubstituted arenes as substrates for this enzyme. Furthermore, it is hoped that the work presented has demonstrated the versatility of the metabolites of dioxygenase enzymes, by reporting the first total synthesis of pleiogenone A, and approaches towards tetrodotoxin.

Further study of the substrate scope and selectivity of toluene dioxygenase will identify new metabolites with potential synthetic utility, and will further refine Boyd's model[72] for the selectivity of the enzymatic dihydroxylation. Any further investigation of the selectivity of the toluene dioxygenase-mediated dihydroxylation should attempt to address the origin of the selectivity observed with alkynyl substrates. The could potentially be accomplished through the crystallization of the enzyme with bound alkynyl substrate. To understand the structure-activity relationship of the pleiogenones with respect to their anti-proliferative activity, the described synthetic sequence will be applied in the production of pleiogenone analogues for biological testing. Research continues on the formal synthesis of tetrodotoxin through the described approach.

In a continued effort to develop versatile methods for the synthesis of polyhydroxylated cyclohexeneone natural products, research towards the synthesis of xylosmin (**156**) is ongoing. The current synthetic route (Scheme 39) has led to the

production of enone **372**, which can be deprotected to afford the polyhydroxylated cyclohexeneone fragment of xylosmin (**156**).



Scheme 39: Current synthetic approach towards xylosmin (**156**).

5. Experimental Section

General experimental

All non-hydrolytic reactions were carried out under an argon atmosphere. Glassware used for moisture-sensitive reactions was flame-dried under vacuum and subsequently purged with argon. THF was distilled from sodium/benzophenone. Methylene chloride and acetonitrile were distilled from calcium hydride. Flash column chromatography was performed using Silicycle SiliaFlash P60 silica gel (40–66 μm). Analytical thin-layer chromatography was performed using silica gel 60-F_{254} plates. Melting points were measured on a Thomas-Hoover melting point apparatus and are reported uncorrected. IR spectra were obtained on a Perkin-Elmer FT-IR 1600 Series Spectrum One instrument. ^{1}H and ^{13}C NMR spectra were obtained on either a 300 MHz (75 MHz) or 600 MHz (150 MHz) Bruker spectrometer. Data are reported as (s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, m = multiplet, br = broad; coupling constants(s) in Hz, integration). Specific rotation measurements are given in deg cm^3 g^{-1} dm^{-1} and were recorded on a Perkin-Elmer 341 Polarimeter. Large-scale fermentation was performed in a 15-L B. Braun Biostat C-15 fermenter. Combustion analyses were performed by Atlantic Microlabs, Norcross, Georgia, USA. Chiral HPLC was carried out on an Agilent 1100 series instrument equipped with a UV detector monitoring at 254 nm and an ODH chiral column. HPLC flow-rate was 0.5 mL/ min using a gradient from 95:5 hexane/isopropanol to 5:95 hexane/isopropanol over 20 min (Condition A); or 90:10 hexane/iso-propanol to 60:40 hexanes/iso-propanol over 20 min (Condition B).

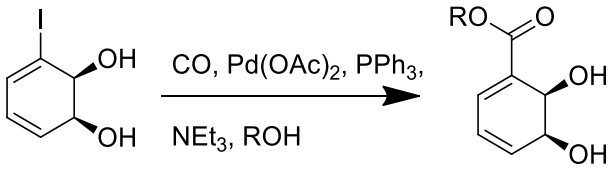
General procedure for diimide reductions with potassium azodicarboxylate

To a stirred solution of diene (2.5 mmol) and potassium azodicarboxylate (PAD) (7.5 to 15.0 mmol) in MeOH (4 mL), glacial acetic acid was added (17.5 to 37.5 eq.) dropwise at -15 °C. The reaction was allowed to warm to room temperature over 14 h, then quenched by the addition of saturated aqueous solution Na_2CO_3 (7 to 15 mL of a saturated aqueous solution), concentrated under reduced pressure and extracted with ethyl acetate (5 x 5 mL). The combined organic layers were washed with brine (1 x 7 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude material was recrystallized from ethyl acetate/pentane.

General procedure for the transesterification of PAD-reduced metabolites

To a stirred solution of an ester (0.5 mmol) in MeOH (5 mL) was added NaOMe until the solution was observed to be just basic by pH paper. Reaction mixture was allowed to stir at room temperature until the starting material was consumed by TLC. Product was purified by flash column chromatography (3:2 v/v ethyl acetate/hexanes).

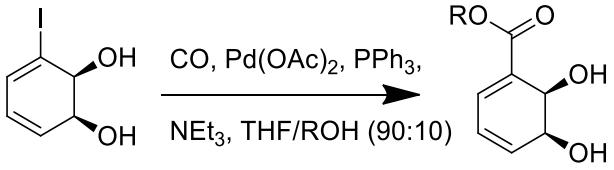
General procedure for carbonylation of diol 78 to corresponding esters (83, 310-312)



A solution of **78**[162] (500 mg, 2.10 mmol) was dissolved in alcohol (30 mL), and carbon monoxide was bubbled through the reaction mixture for 15 minutes, after which triethylamine (0.59 mL, 4.2 mmol) was added followed by solid triphenylphosphine (110

mg, 0.42 mmol) and palladium (II) acetate (47 mg, 0.21 mmol). The orange suspension was heated to 40 ºC. The reaction was monitored by TLC [hexanes/EtOAc (1:1)] and consumption of the starting material was observed after 1-2.5 hours. Upon cooling to room temperature, the crude reaction mixture was concentrated to dryness under reduced pressure. The residue was then dissolved in diethyl ether, filtered through a plug of Celite, and concentrated. The crude material was purified by flash column chromatography [hexanes/EtOAc (1:1)] to yield the corresponding ester.

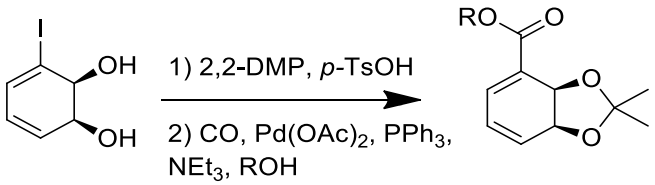
General procedure for carbonylation of diol 78 to corresponding esters (313, 314, 327, 328)



A solution of **78**[162] (500 mg, 2.10 mmol) was dissolved in a solution of THF/alcohol (9:1) (30 mL), and carbon monoxide was bubbled through the reaction mixture for 15 minutes, after which triethylamine (0.59 mL, 4.2 mmol) was added followed by solid triphenylphosphine (110 mg, 0.42 mmol) and palladium (II) acetate (47 mg, 0.21 mmol). The orange suspension was heated to 40 ºC. The reaction was monitored by TLC [hexanes/EtOAc (1:1)] and consumption of the starting material was observed after 4-6 hours. Upon cooling to room temperature, the crude reaction mixture was concentrated to dryness under reduced pressure. The residue was then dissolved in diethyl ether,

filtered through a plug of Celite, and concentrated. The crude material was purified by flash column chromatography [hexanes/EtOAc (1:1)] to yield the corresponding ester.

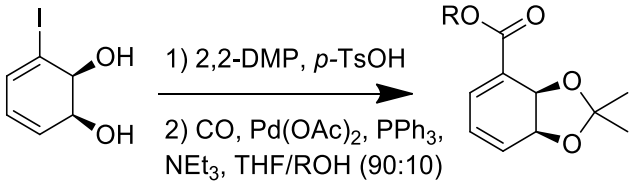
General procedure for the acetonide protection of diol 78 and subsequent carbonylation to corresponding esters (315, 317, 321, 322)



A solution of **78**[162] (500 mg, 2.10 mmol) was dissolved in 2,2-dimethoxypropane (10 mL), to which was added a catalytic amount of *p*-toluenesulfonic acid. The reaction was monitored by thin layer chromatography (TLC), and was observed to be complete after 0.5 hours. The reaction mixture was concentrated under reduced pressure, diluted with EtOAc (15 mL) and washed with a saturated solution of sodium bicarbonate (2 x 3 mL) and brine (1 x 2 mL). The reaction mixture was concentrated under reduced pressure, and dissolved in alcohol (30 mL). Carbon monoxide was bubbled through the reaction mixture for 15 minutes, after which triethylamine (0.59 mL, 4.2 mmol) was added followed by solid triphenylphosphine (110 mg, 0.42 mmol) and palladium (II) acetate (47 mg, 0.21 mmol). The orange suspension was heated to 40 ºC. The reaction was monitored by TLC [hexanes/EtOAc (4:1)] and consumption of the starting material was observed after 1-2.5 hours. Upon cooling to room temperature, the crude reaction mixture was concentrated to dryness under reduced pressure. The residue was then dissolved in diethyl ether, filtered through a plug of Celite, and concentrated under

reduced pressure. The crude material was purified by flash column chromatography [hexanes/EtOAc (9:1)] to yield the corresponding ester.

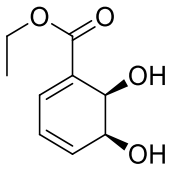
General procedure for the acetonide protection of diol 78 and subsequent to corresponding esters (323-326)



A solution of **6**[10] (500 mg, 2.10 mmol) was dissolved in 2,2-dimethoxypropane (10 mL), to which was added a catalytic amount of *p*-toluenesulfonic acid. The reaction was monitored by thin layer chromatography (TLC), and was observed to be complete after 0.5 hours. The reaction mixture was concentrated under reduced pressure, diluted with EtOAc (15 mL) and washed with a saturated solution of sodium bicarbonate (2 x 3 mL) and brine (1 x 2 mL). The reaction mixture was concentrated under reduced pressure, and dissolved in a solution of THF/alcohol (9:1) (30 mL). Carbon monoxide was bubbled through the reaction mixture for 15 minutes, after which triethylamine (0.59 mL, 4.2 mmol) was added followed by solid triphenylphosphine (110 mg, 0.42 mmol) and palladium (II) acetate (47 mg, 0.21 mmol). The orange suspension was heated to 40 ºC. The reaction was monitored by TLC [hexanes/EtOAc (4:1)] and consumption of the starting material was observed after 4-6 hours. Upon cooling to room temperature, the crude reaction mixture was concentrated to dryness under reduced pressure. The residue was then dissolved in diethyl ether, filtered through a plug of Celite, and concentrated

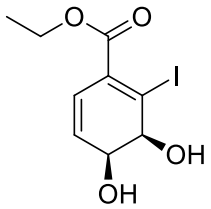
under reduced pressure. The crude material was purified by flash column chromatography [hexanes/EtOAc (9:1)] to yield the corresponding ester.

(+)-Ethyl (5*S*,6*R*)-5,6-Dihydroxycyclohexa-1,3-dienecarboxylate (83)[66]



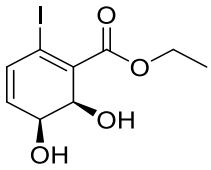
Colourless crystals; R_f = 0.31 [hexanes/EtOAc (1:2)]; mp 49-50 ^{o}C (pentane) [lit.[66] mp 48 ^{o}C (EtOAc/hexanes)]; $[\alpha]_D^{20}$ = +53.5 (*c* = 1.6, $CHCl_3$) [lit.[66] $[\alpha]_D^{23}$ = +54.7 (*c* = 3.8, $CHCl_3$)]; IR (film) *v* 3385, 2981, 2934, 1700, 1280, 1243, 1104, 1068, 825, 771; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.04 (d, *J* = 5.3 Hz, 1H), 6.15 (dt, *J* = 9.4, 1.1 Hz, 1H), 6.03 (dq, *J* = 9.2, 2.3 Hz, 1H), 4.49-4.55 (m, 1H), 4.40-4.48 (m, 1H), 4.22 (q, *J* = 7.0 Hz, 2H), 3.65-3.78 (m, 2H), 1.28 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.1, 138.7, 134.1, 128.7, 122.5, 69.8, 64.5, 60.9, 14.2; MS (EI) *m/z* (%) 184 (9), 166 (20), 138 (26), 122 (33), 121 (52), 105 (100), 77 (39), 51 (21), 45 (20); HRMS (EI) calcd. for $C_9H_{12}O_4$: 184.0736, found: 184.0731; Anal. calcd. for $C_9H_{12}O_4$: C, 58.69; H 6.57. Found C, 58.77; H, 6.60.

Ethyl (3*S*,4*S*)-3,4-dihydroxy-2-iodocyclohex-1,5-dienecarboxylate (210)



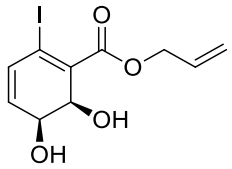
Off-white solid; mp 105-107 °C (pentane); $[\alpha]_D^{20}$ = -72.4 (c = 0.2, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.19 (d, *J* = 9.8 Hz, 1H), 6.13 (dd, *J* = 9.6, 3.7 Hz, 1H), 4.44 (brd, 1H), 4.39 (brd, 1H), 4.32 (q, *J* = 7.2 Hz, 2H), 1.37 (t, *J* = 7.1 Hz, 3H).

Ethyl (5*S*,6*R*)-5,6-dihydroxy-2-iodocyclohex-1,3-dienecarboxylate (211)



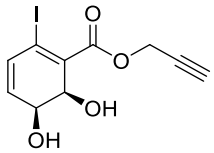
Off-white solid; mp 107-110 °C (pentane); $[\alpha]_D^{20}$ = 67.1 (c = 0.45, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.47 (d, *J* = 9.3 Hz, 1H), 5.86 (d, *J* = 9.4 Hz, 1H), 4.55 (m, 1H), 4.48 (m, 1H), 4.34 (m, 2H), 1.39 (t, *J* = 7.1 Hz, 3H).

Allyl (5*S*,6*R*)-5,6-dihydroxy-2-iodocyclohex-1,3-dienecarboxylate (215)



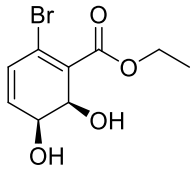
Off-white solid; mp 63-66 °C (pentane); $[\alpha]_D^{20}$ = 68.0 (c = 0.1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.50 (dd, *J* = 9.0, 2.3 Hz, 1H), 6.02 (m, 1H), 5.88 (ddd, *J* = 9.8, 1.9, 1.3 Hz, 1H), 5.43 (ddd, *J* = 17.2, 1.5, 1.4 Hz, 1H), 5.33 (dd, *J* = 10.4, 1.2 Hz, 1H), 4.79 (m, 2H), 4.58 (d, *J* = 5.6 Hz, 1H), 4.51 (brd, 1H).

Propargyl (5*S*,6*R*)-5,6-dihydroxy-2-iodocyclohex-1,3-dienecarboxylate (217)



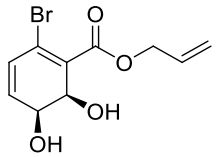
Off-white solid; mp 70-72 °C (pentane); $[\alpha]_D^{20}$ = 77.1 (c = 0.5, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.51 (dd, *J* = 9.8, 2.4 Hz, 1H), 5.89 (ddd, *J* = 9.8, 1.8, 1.0 Hz, 1H), 4.87 (m, 1H), 4.59 (t, *J* = 5.9 Hz, 1H), 4.50 (m, 2H), 2.56 (t, *J* = 2.4 Hz, 1H).

Ethyl (5*S*,6*R*)-5,6-dihydroxy-2-bromocyclohex-1,3-dienecarboxylate (224)



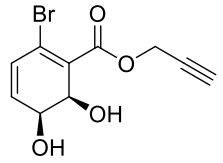
White solid; mp 78-80 °C (pentane); $[\alpha]_D^{20}$ = 46.1 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.19 (dd, *J* = 9.9, 2.3 Hz, 1H), 6.06 (ddd, *J* = 9.8, 1.9, 1.2 Hz, 1H), 4.59 (m, 1H), 4.51 (m, 1H), 4.35 (m, 2H), 1.38 (t, *J* = 7.1 Hz, 3H).

Allyl (5*S*,6*R*)-5,6-dihydroxy-2-bromocyclohex-1,3-dienecarboxylate (228)



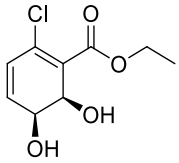
White solid; mp 59-61 °C (pentane); $[\alpha]_D^{20}$ = 52.3 (c = 0.7, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.20 (dd, *J* = 9.9, 2.4 Hz, 1H), 6.08 (ddd, *J* = 8.7, 1.9, 1.4 Hz, 1H), 6.00 (m, 1H), 5.43 (ddd, *J* = 17.3, 2.3, 1.5 Hz, 1H), 5.31 (dd, *J* = 10.4, 1.3 Hz, 1H), 4.78 (m, 2H), 4.63 (d, *J* = 5.9 Hz, 1H), 4.54 (brd, 1H).

Propargyl (5*S*,6*R*)-5,6-dihydroxy-2-bromocyclohex-1,3-dienecarboxylate (230)



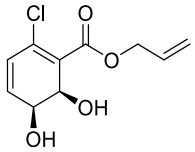
White solid; mp 83-85 °C (pentane); $[\alpha]_D^{20}$ = 65.6 (c = 1.0, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 6.22 (dd, *J* = 9.8, 2.5 Hz, 1H), 6.10 (ddd, *J* = 9.8, 1.9, 1.1 Hz, 1H), 4.88 (d, *J* = 2.6 Hz, 1H), 4.87 (d, J = 2.5 Hz, 1H), 4.64 (dd, *J* = 6.0, 1.2 Hz, 1H), 4.54 (m, 1H), 2.55 (t, *J* = 2.4 Hz, 1H).

Ethyl (5*S*,6*R*)-5,6-dihydroxy-2-chlorocyclohex-1,3-dienecarboxylate (240)



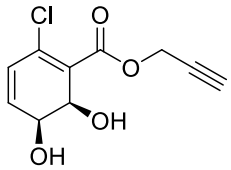
White solid; mp 78-80 °C (pentane); $[\alpha]_D^{20}$ = 46.1 (c = 1.0, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 6.18 (ddd, *J* = 9.8, 1.9, 1.0 Hz, 1H), 6.01 (dd, *J* = 9.8, 2.4 Hz, 1H), 4.64 (dd, *J* = 6.1, 1.4 Hz, 1H), 4.53 (m, 1H), 4.34 (m, 2H), 1.38 (t, *J* = 7.2 Hz, 3H).

Allyl (5*S*,6*R*)-5,6-dihydroxy-2-chlorocyclohex-1,3-dienecarboxylate (244)



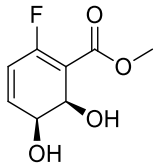
White solid; mp 61-63 °C (pentane); $[\alpha]_D^{20}$ = 33.8 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.16 (dt, *J* = 10.7, 5.2 Hz, 1H), 5.99 (dt, *J* = 16.3, 5.8 Hz, 1H), 5.95 (m, 1H), 5.39 (dd, *J* = 17.2, 1.4 Hz, 1H), 5.27 (dd, *J* = 10.5, 1.5 Hz, 1H), 4.74 (m, 2H), 4.64-4.61 (m, 1H), 4.52-4.47 (m, 1H).

Propargyl (5*S*,6*R*)-5,6-dihydroxy-2-chlorocyclohex-1,3-dienecarboxylate (246)



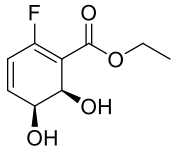
White solid; mp 66-69 °C (pentane); $[\alpha]_D^{20}$ = 29.5 (c = 0.65, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.22 (dt, *J* = 9.8, 1.8 Hz, 1H), 6.03 (dd, *J* = 2.5, 9.9 Hz, 1H), 4.88 (d, *J* = 1.8 Hz, 1H), 4.87 (d, *J* = 1.6 Hz, 1H), 4.68 (d, *J* = 5.9 Hz, 1H), 4.55 (brd, 1H), 2.54 (t, *J* = 2.51 Hz, 1H).

Methyl (5*S*,6*R*)-5,6-dihydroxy-2-fluorocyclohexa-1,3-dienecarboxylate (254)[69]



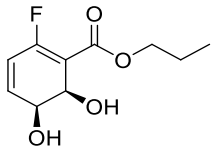
White solid; mp 72-73 °C (pentane), mp 73-75 °C (pentane), mp 73-76 °C (pentane), mp 74-77 °C (pentane) [lit.[69] value: mp 74–76 °C (ethyl acetate)]; R_f = 0.15 [1:1 (hexanes:EtOAc)]; $[\alpha]_D^{20}$ = +68.2 (c = 1.1, MeOH), +69.4 (c = 1.0, MeOH), +70.2 (c = 0.9, MeOH), +72.8 (c = 1.0, MeOH) [lit.[69] value: $[\alpha]_D^{20}$ = +73.2 (c = 1.05, MeOH)]; IR (film) *v* 3558, 3025, 1694, 1439, 1401, 1040; ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.33 (m, 1H), 5.94 (ddd, *J* = 10.2, 8.3, 2.6 Hz, 1H), 4.71 (t, *J* = 6.2 Hz, 1H), 4.55 (m, 1H), 3.83 (s, 3H), 3.17 (brs, 1H), 3.09 (brs, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.0 (d, *J* = 2.2 Hz), 163.2 (d, *J* = 281.0 Hz), 143.1 (d, *J* = 12.1 Hz), 119.6 (d, *J* = 36.2 Hz), 106.2 (d, *J* = 2.2 Hz), 69.06 (s), 67.0 (d, *J* = 6.6 Hz), 52.2 (s); MS (EI) *m/z* (%) 188 (15), 133 (44), 119 (49), 102 (100), 91 (37), 90 (46), 86 (28), 74 (16), 46 (27); HRMS (EI) calcd. for $C_8H_9FO_4^+$ 188.0485, found 188.0484; Anal. calcd. for $C_8H_9FO_4$: C, 51.07; H, 4.82; found: C, 51.18; H, 4.76.

Ethyl (5*S*,6*R*)-5,6-dihydroxy-2-fluorocyclohex-1,3-dienecarboxylate (256)



White solid; mp 70-72 °C (pentane); R_f = 0.24 [2:3 (hexanes:EtOAc)]; $[\alpha]_D^{20}$ = -11.6 (c = 0.65, $CHCl_3$); IR (film) *v* 3418, 2986, 1693, 1601, 1407, 1260, 1138, 1096, 1045, 992, 832; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.34 (m, 1H), 5.96 (ddd, *J* = 10.6, 8.3, 2.7 Hz, 1H), 4.73 (t, *J* = 6.2 Hz, 1H), 4.56 (m, 1H), 4.31 (q, *J* = 7.1 Hz, 2H), 1.35 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.6 (d, *J* = 2.4 Hz), 163.0 (d, *J* = 279.3 Hz), 142.9 (d, *J* = 12.1 Hz), 119.7 (d, *J* = 36.0 Hz), 106.5 (d, *J* = 3.2 Hz), 69.0 (s), 67.1 (d, *J* = 6.1 Hz), 61.3 (s), 14.2 (s); MS (EI) *m/z* (%) 184 (29), 156 (37), 140 (17), 139 (100), 128 (26), 127 (28), 123 (33), 111 (13), 83 (23), 57 (14); HRMS (EI) calcd. for $C_9H_{11}FO_4^+$ 202.0641, found 202.0647; Anal. calcd. for $C_9H_{11}FO_4$: C, 53.47; H, 5.48; found: C, 53.20; H, 5.51.

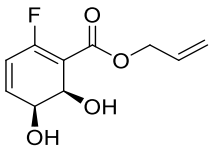
***n*-Propyl (5*S*,6*R*)-5,6-dihydroxy-2-fluorocyclohex-1,3-dienecarboxylate (258)**



White solid; mp 52-54 °C (pentane); R_f = 0.30 [2:3 (hexanes:EtOAc)]; $[\alpha]_D^{20}$ = -11.3 (c = 0.55, $CHCl_3$); IR (film) *v* 3390, 2970, 1693, 1600, 1407, 1266, 1137, 1095, 1042, 993, 938, 829; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.34 (m, 1H), 5.96 (ddd, *J* = 10.6, 8.2, 2.6 Hz, 1H), 4.73 (t, *J* = 6.4, 1H), 4.57 (brd, 1H), 4.21 (m, 2H), 1.74 (sextet, *J* = 14.2, 7.4 Hz, 2H), 1.00 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.8 (d, *J* = 3.3 Hz), 164.8

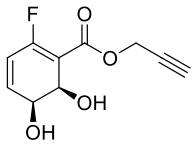
(d, J = 3.4 Hz), 161.1 (s), 142.9 (d, *J* = 12.0 Hz), 119.7 (d, *J* = 36.0 Hz), 106.5 (d, *J* = 2.3 Hz), 69.0 (s), 67.1 (d, *J* = 11.4 Hz), 66.9 (s), 22.0 (s), 10.4 (s); MS (EI) *m/z* (%) 216 (10), 198 (19), 157 (18), 156 (94), 141 (33), 140 (28), 139 (100), 138 (14), 136 (10), 129 (42), 127 (33), 123 (84), 111 (15), 95 (20), 83 (30), 78 (21), 75 (11), 57 (18), 52 (10), 51 (13), 43 (26), 42 (10), 41 (25); HRMS (EI) calcd. for $C_{10}H_{13}FO_4^+$ 216.0798, found 216.0801.

Allyl (5*S*,6*R*)-5,6-dihydroxy-2-fluorocyclohex-1,3-dienecarboxylate (260)



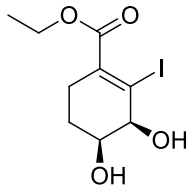
White solid; mp 56-58 °C (pentane); R_f = 0.32 [2:3 (hexanes:EtOAc)]); $[\alpha]_D^{20}$ = -5.4 (c = 0.35, $CHCl_3$); IR (film) *v* 3418, 1694, 1651, 1600, 1404, 1259, 1138, 1095, 1044, 994, 930, 829; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.35 (m, 1H), 5.95 (m, 2H), 5.40 (ddd, *J* = 17.2, 2.3, 1.4 Hz, 1H), 5.28 (dd, *J* = 10.5, 1.5 Hz, 1H), 4.75 (m, 3H), 4.57 (brd, 1H), ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.3 (t, *J* = 2.9 Hz), 161.5 (s), 143.2 (d, *J* = 25.9 Hz), 131.7 (s), 119.7 (d, *J* = 35.8 Hz), 118.4 (s), 106.3 (d, *J* = 2.1 Hz), 69.1 (s), 67.0 (d, *J* = 6.2 Hz), 65.7 (s); MS (EI) *m/z* (%) 214 (15), 157 (17), 156 (100), 155 (27), 139 (56), 128 (77), 127 (91), 123 (20), 111 (14), 107 (17), 100 (12), 99 (30), 83 (38), 71 (14), 53 (10), 51 (13), 41 (39); HRMS (EI) calcd. for $C_{10}H_{11}FO_4^+$ 214.0641, found 214.0642.

Propargyl (5*S*,6*R*)-5,6-dihydroxy-2-fluorocyclohex-1,3-dienecarboxylate (262)



White solid; mp 82-84 °C (pentane); R_f = 0.27 [2:3 (hexanes:EtOAc)]; $[\alpha]_D^{20}$ = +13.4 (c = 1.7, $CHCl_3$); IR (film) *v* 3296, 2949, 1703, 1654, 1599, 1405, 1256, 1135, 1095, 1045, 991, 828; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.35 (m, 1H), 5.95 (ddd, *J* = 10.6, 8.3, 2.8 Hz, 1H), 4.82 (d, *J* = 2.5, 2H), 4.72 (t, *J* = 6.3 Hz, 1H), 4.56 (brd, 1H), 2.51 (t, *J* = 2.6 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.8 (s), 164.6 (d, *J* = 2.6 Hz), 162.1 (s), 143.9 (d, *J* = 12.7 Hz), 119.6 (d, *J* = 35.8 Hz), 105.8 (d, *J* = 2.1 Hz), 77.4 (s), 75.3 (s), 69.1 (s), 66.9 (d, *J* = 5.7 Hz), 52.5 (s); MS (EI) *m/z* (%) 194 (19), 156 (19), 140 (11), 139 (100), 128 (11), 127 (14), 123 (54), 111 (13), 95 (13), 83 (27), 57 (19), 44 (13); HRMS (EI) calcd. for $C_9H_{13}ClO_4^+$ 212.0485, found 212.0482; Anal. calcd. for $C_9H_{13}BrO_4$: C, 56.61; H, 4.28; found: C, 56.87; H, 4.42.

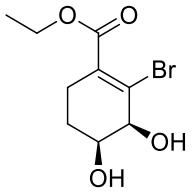
Ethyl (3*S*,4*S*)-3,4-dihydroxy-2-iodocyclohex-1-enecarboxylate (263)



Off-white solid; mp 108-109 °C (pentane); R_f = 0.46 [2:3 (hexanes:EtOAc)]; $[\alpha]_D^{20}$ = -32.6 (c = 0.75, $CHCl_3$); IR (film) *v* 3270, 2940, 1704, 1593, 1463, 1253; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.32 (brd, 1H), 4.30 (q, *J* = 7.1 Hz, 2H), 4.02 (m, 1H), 2.64 (dt, *J* = 18.1,

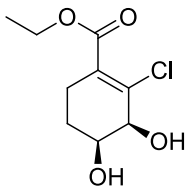
5.7 Hz, 1H), 2.39 (dt, *J* = 17.9, 6.7 Hz, 1H), 1.94 (m, 2H), 1.37 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.0,140.7, 102.7, 74.8, 68.2, 61.7, 27.2, 25.3, 14.1; MS (EI) *m/z* (%) 312 (40), 266 (85), 231 (18), 222 (96), 194 (73), 185 (90), 167 (20), 165 (21), 139 (100), 121 (20), 111 (61), 93 (42), 83 (53), 67 (38), 65 (38), 55 (24), 53 (21); HRMS (EI) calcd. for $C_9H_{13}IO_4^+$ 311.9859, found 311.9847.

Ethyl (3*S*,4*S*)- 3,4-dihydroxy-2-bromocyclohex-1-enecarboxylate (264)



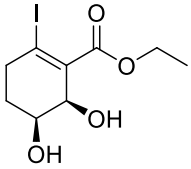
White solid; mp 90-92 °C (pentane); R_f = 0.26 [2:3 (hexanes:EtOAc)]; $[\alpha]_D^{20}$ = -52.9 (c = 1.0, $CHCl_3$); IR (film) *v* 3255, 2950, 1708, 1461, 1252, 1094; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.31 (brd, 1H), 4.29 (q, *J* = 7.1 Hz, 2H), 4.01 (m, 1H), 2.61 (dt, *J* = 17.8, 6.0 Hz, 1H), 2.34 (dt, *J* = 17.9, 6.6 Hz, 1H), 1.95 (m, 1H), 1.86 (m, 2H), 1.35 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.2, 134.7, 124.1, 72.2, 68.3, 61.6, 26.5, 25.2, 14.1; MS (EI) *m/z* (%) 223 (12), 222 (27), 221 (21), 220 (30), 219 (12), 185 (38), 183 (12), 177 (18), 176 (22), 175 (21), 174 (21), 141 (100), 102 (21), 113 (12), 111 (14), 95 (61), 67 (21), 66 (13), 65 (17), 55 (11), 53 (14), 44 (16), 43 (10); HRMS (EI) 263.9997 = $C_9H_{13}BrO_4^+$; calcd. $C_9H_{13}BrO_4^+$ $[(M-1)^+, H]$: 262.9919; found: 262.9925; Anal. calcd. for $C_9H_{13}BrO_4$: C, 40.78; H, 4.94; found: C, 40.57; H, 4.87.

Ethyl (3*S*,4*S*)-3,4-dihydroxy-2-chlorocyclohex-1-enecarboxylate (265)



White solid; mp 68-70 °C (pentane); R_f = 0.30 [2:3 (hexanes:EtOAc)]; $[\alpha]_D^{20}$ = -62.3 (c = 0.4, $CHCl_3$); IR (film) *v* 3268, 2952, 1726, 1624, 1464, 1368, 1289, 1254, 1094, 940; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.29 (q, *J* = 7.0 Hz, 2H), 4.25 (br s, 1H), 4.02 (br s, 1H), 2.65 (dt, *J* = 18.0, 5.9 Hz, 1H), 2.38 (dt, *J* = 18.0, 6.5 Hz, 1H), 1.95 (m, 1H), 1.83 (m, 1H), 1.35 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.5, 133.3, 131.1, 71.1, 68.1, 61.4, 25.4, 25.2, 14.1; MS (EI) *m/z* (%) 185 (14), 178 (28), 177 (28), 176 (84), 175 (21), 157 (17), 139 (11), 133 (13), 132 (37), 131 (41), 130 (100), 104 (19), 102 (50), 67 (27), 65 (24), 55 (10), 53 (18), 51 (11), 43 (12); HRMS (EI) calcd. for $C_9H_{13}ClO_4^+$ 220.0402, found 220.0505.

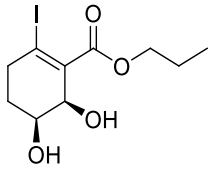
Ethyl (5*S*,6*R*)-5,6-dihydroxy-2-iodocyclohex-1-enecarboxylate (266)



Off-white solid; mp 106-108 °C (pentane); R_f = 0.42 [2:3 (hexanes:EtOAc)]; $[\alpha]_D^{20}$ = -31.6 (c = 0.45, $CHCl_3$); IR (film) *v* 3282, 2950, 1714, 1642, 1461, 1241; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.52 (brd, 1H), 4.36 (q, *J* = 7.1 Hz, 2H), 3.95 (m, 1H), 3.00 (dt, *J* = 19.1, 5.4 Hz, 1H), 2.75 (dt, *J* = 18.6, 6.9 Hz, 1H), 1.98 (m, 1H), 1.77 (m, 1H), 1.37 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.0, 136.7, 108.5, 68.4, 67.1, 61.7, 41.1, 28.4,

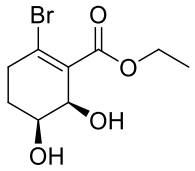
14.1; MS (EI) *m/z* (%) 312 (12), 294 (61), 278 (31), 276 (40), 269 (37), 267 (46), 249 (43), 248 (30), 231 (62), 223 (72), 185 (99), 142 (73), 141 (100), 139 (96), 113 (61), 111 (92), 95 (90), 83 (41), 79 (40), 66 (65), 65 (59), 53 (46); HRMS (EI) calcd. for $C_9H_{13}IO_4^+$ 311.9859, found 311.9859.

_n_-Propyl (5*S*,6*R*)-5,6-dihydroxy-2-iodocyclohex-1-enecarboxylate (267)



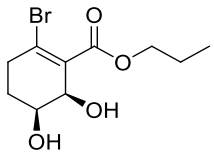
Off-white solid; mp 70-72 °C (pentane); R_f = 0.43 [2:3 (hexanes:EtOAc)]; $[\alpha]_D^{20}$ = -42.6 (c = 0.5, $CHCl_3$); IR (film) *v* 3271, 2962, 2879, 1715, 1606, 1462, 1231; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.50 (brd, 1H), 4.23 (td, *J* = 6.5, 1.9 Hz, 2H), 3.94 (m, 1H), 3.01 (dt, *J* = 19.1, 5.5 Hz, 1H), 2.77 (dt, *J* = 19.0, 7.1 Hz, 1H), 1.97 (m, 1H), 1.77 (m, 2H), 1.74 (m, 2H), 1.04 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.0, 136.7, 108.5, 68.4, 67.3, 67.1, 41.2, 28.4, 21.9, 10.6; MS (EI) *m/z* (%) 326 (10), 304 (10), 291 (20), 290 (13), 199 (38), 155 (44), 139 (16), 132 (15), 113 (39), 111 (21), 95 (33), 69 (100), 44 (26), 43 (11), 41 (10); HRMS (EI) calcd. for $C_{10}H_{15}IO_4^+$ 326.0015, found 326.0015.

Ethyl (5*S*,6*R*)-5,6-dihydroxy-2-bromocyclohex-1-enecarboxylate (268)



White solid; mp 87-89 °C (pentane); R_f = 0.24 [2:3 (hexanes:EtOAc)]; $[\alpha]_D^{20}$ = -59.8 (c = 1.0, $CHCl_3$); IR (film) *v* 3269, 2955, 1721, 1622, 1462, 1364, 1241, 1042; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.52 (t, *J* = 3.8 Hz, 1H), 4.32 (q, *J* = 7.2 Hz, 2H), 3.91 (m, 1H), 2.84 (dt, *J* = 19.0, 5.5 Hz, 1H), 2.63 (dt, *J* = 19.0, 7.3 Hz, 1H), 2.01 (m, 1H), 1.81 (m, 1H), 1.36 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.8, 131.7, 131.0, 68.5, 67.0, 61.7, 35.8, 27.1, 14.1; MS (EI) *m/z* (%) 222 (13), 221 (14), 220 (41), 219 (12), 218 (30), 177 (12), 176 (99), 175 (18), 174 (100), 148 (16), 146 (13), 139 (25), 111 (12), 83 (12), 73 (11), 68 (10), 67 (12), 66 (11), 65 (18), 55 (20), 53 (17), 43 (12); HRMS (EI) calcd. for $C_9H_{13}BrO_4^+$ 263.9997, found 263.9996; Anal. calcd. for $C_9H_{13}BrO_4$: C, 40.78; H, 4.94; found: C, 40.71; H, 4.92.

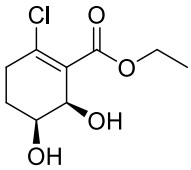
***n*-Propyl (5*S*,6*R*)- 5,6-dihydroxy-2-bromocyclohex-1-enecarboxylate (269)**



White solid; mp 82-85 °C (pentane); R_f = 0.27 [2:3 (hexanes:EtOAc)]; $[\alpha]_D^{20}$ = -49.8 (c = 0.6, $CHCl_3$); IR (film) *v* 3383, 2966, 1714, 1657, 1462, 1426, 1392, 1337, 1268, 1092, 1040, 913; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.53 (t, *J* = 3.8 Hz, 1H), 4.23 (t, *J* = 6.8 Hz,

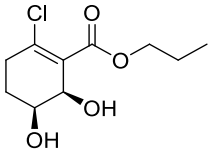
2H), 3.92 (m, 1H), 2.86 (dt, *J* = 19.0, 5.4 Hz, 1H), 2.65 (dt, *J* = 19.1, 7.2 Hz, 1H), 2.03 (m, 1H), 1.82 (m, 1H), 1.77 (sextet, *J* = 7.2 Hz, 2H), 1.02 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.8, 132.3, 130.8, 68.6, 67.3, 67.0, 35.9, 27.1, 21.9, 10.6; MS (EI) *m/z* (%)236 (42), 234 (43), 219 (21), 199 (47), 194 (23), 192 (29), 177 (29), 176 (51), 175 (34), 174 (50), 155 (100), 139 (31), 113 (76), 111 (31), 95 (69), 67 (30), 66 (23), 65 (30), 53 (23), 43 (93), 41 (49); HRMS (EI) calcd. for $C_{10}H_{15}BrO_4^+$ 278.0148, found 278.0169.

Ethyl (5*S*,6*R*)-5,6-dihydroxy-2-chlorocyclohex-1-enecarboxylate (270)



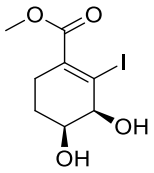
White solid; mp 74-76 °C (pentane); R_f = 0.33 [2:3 (hexanes:EtOAc)]; $[\alpha]_D^{20}$ = -72.3 (c = 0.45, $CHCl_3$); IR (film) *v* 3271, 2958, 1717, 1624, 1461, 1424, 1365, 1241, 1097, 1043; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.54 (s, 1H), 4.36 (q, *J* = 7.1 Hz, 2H), 3.86 (s, 1H), 2.67 (dt, *J* = 19.0, 5.4 Hz, 1H), 2.49 (dt, *J* = 18.7, 7.3 Hz, 1H), 1.99 (m, 1H), 1.79 (m, 1H), 1.33 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.3, 142.2, 127.4, 67.9, 67.1, 61.5, 33.3, 26.0, 14.1; MS (EI) *m/z* (%) 185 (14), 178 (29), 177 (28), 176 (83), 175 (20), 157 (18), 139 (14), 133 (14), 132 (38), 131 (41), 130 (100), 104 (19), 102 (49), 67 (25), 65 (23), 55 (10), 53 (16), 51 (10), 44 (11), 43 (11); HRMS (EI) calcd. for $C_9H_{13}ClO_4^+$ 220.0402, found 220.0405; Anal. calcd. for $C_9H_{13}ClO_4$: C, 48.99; H, 5.94; found: C, 48.77; H, 5.91.

***n*-Propyl (5*S*,6*R*)-5,6-dihydroxy-2-chlorocyclohex-1-enecarboxylate (271)**



White solid; mp 60-63 °C (pentane); R_f = 0.34 [2:3 (hexanes:EtOAc)]; $[\alpha]_D^{20}$ = -65.6 (c = 0.3, $CHCl_3$); IR (film) *v* 3420, 3304, 2964, 1714, 1661, 1552, 1521, 1392, 1344, 1271, 1094, 1056, 1009, 928, 795; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.58 (m, 1H), 4.24 (t, *J* = 6.72 Hz, 2H), 3.90 (m, 1H), 2.73 (dt, *J* = 18.8, 5.7 Hz, 1H), 2.55 (dt, *J* = 18.4, 7.3 Hz, 1H), 2.03 (m, 1H), 1.85 (m, 1H), 1.77 (sextet, *J* = 7.1 Hz, 2H), 1.02 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.4, 143.0, 127.1, 68.0, 67.2, 67.1, 33.6, 26.1, 21.9, 10.6; MS (EI) *m/z* (%) 199 (14), 192 (20), 191 (15), 190 (57), 175 (25), 157 (12), 150 (25), 149 (14), 148 (76), 133 (13), 132 (35), 131 (40), 130 (100), 104 (13), 102 (32), 67 (15), 65 (16), 43 (49), 41 (19); HRMS (EI) calcd. for $C_{10}H_{15}ClO_4^+$ 234.0659, found 234.0661.

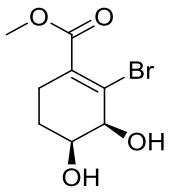
Methyl (3*S*,4*S*)-3,4-dihydroxy-2-iodocyclohex-1-enecarboxylate (272)



White solid; mp 78-80 °C (pentane) [lit.[90] mp 80-81 °C (hexanes)]; R_f = 0.23 [2:3 (hexanes:EtOAc)]; $[\alpha]_D^{20}$ = -32.5 (c = 0.1, $CHCl_3$) [lit.[90] $[\alpha]_D^{20}$ = -34.5 (c = 1.1, $CHCl_3$)]; IR (film) *v* 3259, 2949, 1722, 1635, 1433, 1347, 1094; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.33 (br s, 1H), 4.02 (m, 1H), 3.83 (s, 3H), 2.65 (dt, *J* = 18.0, 5.7 Hz, 1H), 2.35 (dt, *J* = 18.0, 5.7 Hz, 1H), 1.97 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.5, 136.8, 108.5, 68.4,

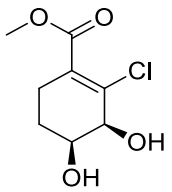
67.1, 52.3, 41.0, 28.3; MS (EI) *m/z* (%) 314 (16), 299 (14), 298 (100), 296 (13), 294 (22); HRMS (EI) calcd. for $C_8H_{11}IO_4^+$ 297.9702, found 297.9713.

Methyl (3*S*,4*S*)-3,4-dihydroxy-2-bromocyclohex-1-enecarboxylate (273)



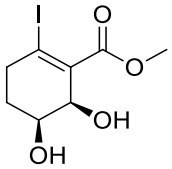
White solid; mp 91-93 °C (pentane) [lit.[90] mp 92-93 °C (pentane)]; R_f = 0.42 [1:2 (hexanes:EtOAc)]; $[\alpha]_D^{20}$ = -30.6 (c = 0.7, $CHCl_3$), [lit.[90] $[\alpha]_D^{20}$ = -32.4 (c = 0.7, $CHCl_3$)]; IR (film) *v* 3270, 2951, 1728, 1431, 1254, 1092, 756; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.29 (d, *J* = 3.9 Hz, 1H), 4.01 (dt, *J* = 8.8, 3.5 Hz, 1H), 3.81 (s, 3H), 2.61 (dt, *J* = 17.8, 5.6 Hz, 1H), 2.35 (dt, *J* = 18.0, 6.7 Hz, 1H), 1.85-1.96 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.5, 134.4, 124.8, 72.2, 68.2, 52.3, 26.4, 25.2; MS (EI) *m/z* (%) 220 (30), 218 (33), 208 (15), 206 (16), 177 (10), 176 (98), 175 (15), 174 (100), 148 (21), 146 (18), 139 (14), 83 (11), 65 (16), 59 (29), 55 (17), 53 (17); HRMS (EI) calcd. for $C_8H_{11}BrO_4^+$ 249.9841, found 249.9852.

Methyl (3*S*,4*S*)-3,4-dihydroxy-2-chlorocyclohex-1-enecarboxylate (274)



White solid; mp 91-93 °C (pentane) [lit.[90] mp 92-94 °C (pentane)]; R_f = 0.23 [2:3 (hexanes:EtOAc)]; $[\alpha]_D^{20}$ = -59.7 (c = 0.2, $CHCl_3$) [lit.[90] $[\alpha]_D^{20}$ = -62.3 (c = 0.4, $CHCl_3$)]; IR (film) *v* 3400, 2953, 1728, 1627, 1435, 1344, 1259, 1143, 1091, 985, 931; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.24 (brd, 1H), 4.00 (m, 1H), 3.82 (s, 3H), 2.64 (dt, *J* = 18.1, 5.7 Hz, 1H), 2.38 (dt, *J* = 18.0, 6.4 Hz, 1H), 1.94 (m, 1H), 1.83 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.9, 134.0, 130.7, 71.1, 68.1, 52.2, 25.4, 25.2; MS (EI) *m/z* (%) 175 (12), 171 (17), 164 (34), 163 (26), 162 (100), 139 (12), 133 (11), 132 (31), 131 (35), 130 (88), 104 (14), 102 (38), 67 (17); HRMS (EI) calcd. for $C_8H_{11}ClO_4^+$ 206.0346, found 206.0350; Anal. calcd. for $C_8H_{11}ClO_4$: C, 46.50; H, 5.37; found: C, 46.69; H, 5.44.

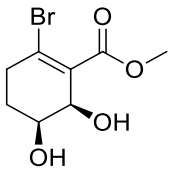
Methyl (5*S*,6*R*)-5,6-dihydroxy-2-iodocyclohex-1-enecarboxylate (275)[90]



White solid; mp 83-85 °C (pentane), mp 84-86 °C (pentane) [lit.[90] mp 85-86 °C (hexanes)]; R_f = 0.27 [2:3 (hexanes:EtOAc)]; $[\alpha]_D^{20}$ = -37.4 (c = 0.2, $CHCl_3$), $[\alpha]_D^{20}$ = -38.0 (c = 0.2, $CHCl_3$) [lit.[90] $[\alpha]_D^{20}$ = -39.5 (c = 1.1, $CHCl_3$)]; IR (film) *v* 3268, 2950, 1727, 1634, 1432, 1236, 1093; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.50 (br s, 1H), 3.94 (m, 1H),

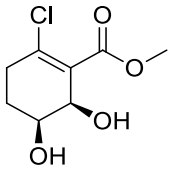
3.86 (s, 3H), 3.03 (dt, *J* = 19.9, 5.5 Hz, 2H), 2.77 (dt, *J* = 19.1, 7.1 Hz, 2H), 1.97 (m, 1H), 1.74 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.2, 140.4, 103.2, 74.8, 68.2, 52.4, 27.2, 25.3; MS (EI) *m/z* (%) 314 (48), 312 (51), 298 (100), 294 (24), 280 (38), 262 (36), 231 (35), 223 (37), 171 (98), 153 (24), 139 (97), 127 (57), 125 (69), 111 (72), 95 (88), 83 (30), 67 (36), 53 (29); HRMS (EI) calcd. for $C_8H_{11}IO_4^+$ 297.9702, found 297.9707.

Methyl (5*S*,6*R*)- 5,6-dihydroxy-2-bromocyclohex-1-enecarboxylate (276)



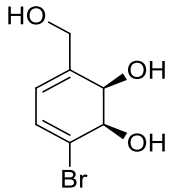
White solid; mp 95-97 °C (pentane), mp 98-100 °C (pentane) [lit.[90] mp 98-100 °C (pentane)]; R_f = 0.36 [1:2 (hexanes:EtOAc)]; $[\alpha]_D^{20}$ = -43.5 (c = 0.7, $CHCl_3$), $[\alpha]_D^{20}$ = -52.8 (c = 0.7, $CHCl_3$) [lit.[90] $[\alpha]_D^{20}$ = -44.9 (c = 0.7, $CHCl_3$)]; IR (film) *v* 3237, 2953, 1731, 1430, 1217, 1099, 1037; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.53 (s, 1H), 3.90-3.93 (m, 1H), 3.86 (s, 3H), 2.86 (dt, *J* = 19.0, 5.7 Hz, 1H), 2.65 (dt, *J* = 19.4, 7.2 Hz, 1H), 2.05-2.08 (m, 1H), 1.85-1.86 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.1, 132.5, 130.7, 68.5, 67.0, 52.3, 36.8, 27.1; MS (EI) *m/z* (%) 209 (13), 208 (31), 207 (14), 206 (32), 177 (17), 176 (20), 175 (19), 174 (20), 171 (27), 139 (13), 127 (100), 95 (59), 67 (22), 66 (10), 65 (13), 53 (11), HRMS (EI) calcd. for $C_8H_{11}BrO_4^+$ 249.9841, found 249.9832; Anal. calcd. for $C_8H_{11}BrO_4$: C, 38.27; H, 4.42; found: C, 38.33; H, 4.34.

Methyl (5*S*,6*R*)-5,6-dihydroxy-2-chlorocyclohex-1-enecarboxylate (277)



Off-white solid; mp 74-75 °C (pentane), mp 74-76 °C (pentane) [lit.[90] mp 75-77 °C (pentane)]; R_f = 0.18 [2:3 (hexanes:EtOAc)]; $[\alpha]_D^{20}$ = -65.9 (c = 0.8, $CHCl_3$), $[\alpha]_D^{20}$ = -66.5 (c = 1.0, $CHCl_3$) [lit.[90] $[\alpha]_D^{20}$ = -68.5 (c = 1.0, $CHCl_3$)]; IR (film) *v* 3228, 2956, 1731, 1430, 1336, 1245, 1101, 1040, 967, 931; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.56 (brd, 1H), 3.87 (m, 4H), 2.69 (dt, *J* = 19.0, 5.3 Hz, 1H), 2.51 (dt, *J* = 19.0, 7.3 Hz, 1H), 2.02 (m, 1H), 1.82 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.7, 142.6, 127.3, 67.9, 67.2, 52.3, 33.4, 26.0; MS (EI) *m/z* (%) 176 (10), 175 (12), 174 (29), 162 (17), 132 (35), 131 (13), 130 (100), 102 (16), 59 (14), HRMS (EI) calcd. for $C_9H_{13}ClO_4^+$ 206.0346, found 206.0352; Anal. calcd. for $C_9H_{13}ClO_4$: C, 46.50; H, 5.37; found: C, 46.63; H, 5.40.

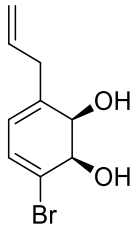
(1*R*,2*R*)-3-Bromo-6-(hydroxymethyl)cyclohexa-3,5-diene- 1,2-diol (301)



White powder; mp 94-96 °C (pentane); $[\alpha]_D^{20}$ = -8.3 (c = 1.0, MeOH); IR ($CHCl_3$) *v* 3337, 2920, 2861, 1648, 1579, 1388, 1333, 1096, 1014, 842; ^{1}H NMR (300 MHz, MeOD) δ 6.38 (d, *J* = 6.0 Hz, 1H), 5.80 (ddd, *J* = 6.1, 3.0, 1.6 Hz, 1H), 4.38 (dd, *J* = 6.0, 0.8 Hz, 1H), 4.24–4.14 (m, 3H); ^{13}C NMR (75 MHz, MeOD) δ 142.3, 127.9, 126.5, 119.2, 74.1, 71.2, 63.0; MS (EI) *m/z* (%) 204 (73), 202 (83), 186 (46), 185 (30), 184 (32), 177 (26),

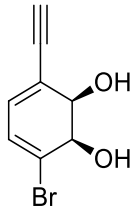
162 (31), 123 (28), 109 (49), 107 (26), 105 (34), 95 (74), 94 (86), 79 (28), 78 (27), 77 (100), 67 (25), 65 (32), 63 (26), 61 (25), 55 (32), 53 (25), 51 (35), 44 (58), 43 (69), 41 (29); HRMS (EI) calcd. for $C_7H_9BrO_3^+$ 219.9735, found 219.9731; HPLC Condition B (minor enantiomer rt = 11.4 min, major enantiomer rt = 10.0 min).

(1*R*,2*R*)-3-Allyl-6-bromocyclohexa-3,5-diene-1,2-diol (302)



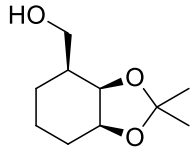
Off-white amorphous powder; R_f = 0.37 [1:1 (hexanes:EtOAc)]; mp 128-130 ^oC (MeOH/Et_2O); $[\alpha]_D^{20}$ = +13.6 (c = 0.86, MeOH); IR (MeOH) *v* 3429, 2095, 1642, 1424, 1286, 1097, 1079, 1049, 1018, 913, 838; ^1H NMR (300 MHz, $CDCl_3$) δ 6.35 (d, *J* = 6.1 Hz, 1H), 5.87 (ddt, *J* = 16.9, 10.1, 6.7 Hz, 1H), 5.63 (td, *J* = 6.0, 1.4, Hz, 1H), 5.18-5.09 (m, 2H), 4.32 (brs, 2H), 2.97 (ddd, *J* = 6.5, 2.6, 1.5 Hz, 2H); ^{13}C NMR (75 MHz, CDCl3) δ 140.0, 135.0, 127.1, 123.9, 119.7, 117.3, 72.8, 71.0, 37.3; MS (EI) *m/z* (%) 270 (24), 254 (21), 252 (37), 250 (20), 212 (37), 210 (37), 185 (22), 184 (97), 183 (100), 182 (97), 181 (76), 157 (98), 155 (91), 142 (21), 110 (73), 109 (20), 103 (27), 102 (78), 90 (21), 82 (26), 76 (55), 75 (60), 63 (28), 51 (46), 50 (48); HRMS (EI) calcd. for $C_9H_{11}BrO_2^+$ 229.9942, found 229.9945; HPLC Condition A (minor enantiomer rt = 4.1 min, major enantiomer rt = 7.0 min).

(1*R*,2*R*)-3-Bromo-6-ethynylcyclohexa-3,5-diene-1,2-diol (303)



Off-white solid; mp 118-120 ^{o}C (MeOH/Et_2O); R_f = 0.31 [3:1 (hexanes:EtOAc)]; $[\alpha]_D^{20}$ = +34.6 (c = 0.5, MeOH); IR (film) *v* 3734, 3288, 3200, 2839, 1557, 1414, 1304, 1102, 1085, 1014, 844; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.28 (d, *J* = 6.2 Hz, 1H), 6.05 (d, *J* = 6.1 Hz, 1H), 4.22 (d, *J* = 5.9 Hz, 1H), 4.15 (d, *J* = 6.0 Hz, 1H), 3.24 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 129.8, 127.8, 126.4, 122.1, 82.7, 81.6, 71.8, 66.8; MS (EI) *m/z* (%) 216 (50), 214 (54), 198 (43), 196 (44), 145 (18), 143 (18), 135 (52), 117 (23), 118 (17), 89 (84), 77 (100); HRMS (EI) calcd. for $C_8H_7BrO_2^+$ 213.9629, found 213.9627; HPLC Condition A (minor enantiomer rt = 5.4 min, major enantiomer rt = 10.2 min).

(1*S*, 2*R*, 3*S*)-3-hydroxymethyl-[1,2]-isopropylidenedioxycyclohexane (304)

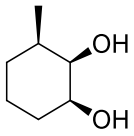


To a degassed solution of acetonide-protected diene diol **301** (800 mg, 3.07 mmol) in methanol (16.0 mL) and triethylamine (3.2 mL) was added Adams' catalyst (PtO_2) (80 mg, 0.35 mmol) and the atmosphere was evacuated. The reaction vessel was charged with hydrogen, and the reaction mixture stirred for 12 h at room temperature. The reaction mixture was then filtered through a pad of celite (MeOH), and the solvent was removed under reduced pressure. The crude residue was purified by flash chromatography [4:1

(hexanes:EtOAc)] to afford cyclohexane **304** as a clear, colourless oil (320 mg, 1.75 mmol, 57 % yield).

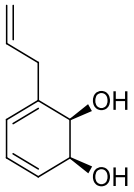
R_f = 0.48 [4:1 (hexanes:EtOAc)]; $[\alpha]_D^{20}$ = -6.13 (c = 2.0, $CHCl_3$); IR ($CHCl_3$) *v* 3691, 3626, 3011, 2940, 2870, 1732, 1451, 1382, 1372, 1235, 1039, 863; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.33 (dd, *J* = 5.3, 3.5 Hz, 1H), 4.16 (td, *J* = 9.0, 5.5 Hz, 1H), 3.83 (dd, *J* = 11.0, 4.3 Hz, 1H), 3.73 (dd, *J* = 11.0, 5.8 Hz, 1H), 1.89-1.72 (m, 3H), 1.54 (s, 3H), 1.58-1.45 (m, 3H), 1.39 (s, 3H) 1.35-1.27 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 108.0, 74.7, 74.5, 60.2, 39.3, 28.4, 27.9, 25.9, 21.5, 20.2; MS (EI) *m/z* (%) 171 (100), 111 (37), 110 (16), 93 (47), 82 (18), 81 (35), 79 (32), 67 (39), 43 (52), 39 (38); HRMS (EI) 186.1256 = $C_{10}H_{18}O_3^+$; calcd. $C_9H_{15}O_3^+$ [(M-15)$^+$, CH_3]: 171.1021; found: 171.1036.

(1*S*,2*R*,3*R*)-3-Methylcyclohexane-1,2-diol (305)[159]



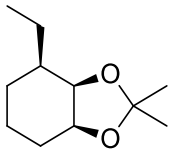
Viscous oil; R_f = 0.32 [95:5 ($CHCl_3$:MeOH)]; $[\alpha]_D$ = –3.7 (c = 1.0, $CHCl_3$) [lit.[159] $[\alpha]_D^{20}$ = –25 (c = 1.1, $CHCl_3$)]; IR (film) *v* 3375, 2929, 975; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.58 (t, *J* = 2.8 Hz, 1H), 3.38 (ddd, *J* = 2.8, 5.8, 11.4 Hz, 1H), 1.87 (ddd, *J* = 2.8, 5.3, 9.1 Hz, 1H), 1.49-1.51 (m, 2H), 1.34-1.37 (m, 2H), 1.08-1.14 (m, 2H), 0.84 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 73.7, 72.6, 35.3, 27.8, 26.6, 23.5, 18.0; MS (EI) *m/z* (%) 130 (20), 112 (67), 97 (100), 71 (63); HRMS (EI) calcd. for $C_7H_{14}O_2^+$ 130.0994, found 130.1012.

***cis*-(1*S*,2*R*)-3-Allylcyclohexa-3,5-diene-1,2-diol (306)**[160]



White solid; mp 38 ^{o}C (hexane/CH_2Cl_2); $[\alpha]_D$ = +10.4 (c = 0.47, MeOH); [lit.[160] $[\alpha]_D^{20}$ = +21 (c = 0.73, $CHCl_3$)]; ^{1}H NMR (300 MHz) δ 5.79–5.98 (m, 3H), 5.74 (d, *J* = 5.0 Hz, 1H), 5.13 (m, 2H), 4.05 (d, *J* = 6.0 Hz, 2H), 3.00 (d, *J* = 6.7 Hz, 2H); MS (EI) *m/z* (%) 152 (8), 134 (100), 91 (72); HRMS (EI) calcd. for $C_9H_{12}O_2^+$ 152.0837, found 152.0834.

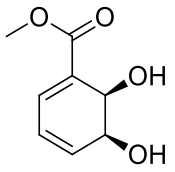
(1*S*,2*R*,3*R*)-3-Ethyl-[1,2]-isopropylidenedioxycyclohexane (307)[161]



Crude (1*S*,2*R*)-3-ethyl-[1,2]-isopropylidenedioxycyclohexa-3,5-diene (98 mg, 0.54 mmol) was dissolved in 2 mL of methanol and 500 µL of triethylamine and the solvent was degassed. Adams' catalyst (10 mg, 0.044 mmol) was then added, and the flask was flushed with hydrogen gas. The reaction vessel was charged with hydrogen and the reaction mixture was stirred for 16 h under the hydrogen atmosphere. The reaction mixture was then filtered through a Celite pad (Et_2O) and the solvent was removed under reduced pressure. The crude mixture was then purified through flash chromatography [9:1 (hexanes:EtOAc)] to provide cyclohexane **307** as a clear, colourless oil (46 mg, 0.25 mmol, 46%).

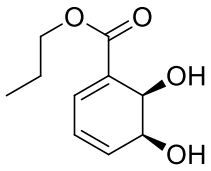
bp 221–223 ^{o}C (760 mmHg); R_f = 0.44 [9:1 (hexanes:EtOAc)]; $[\alpha]_D^{20}$ = +10.9 (c = 1.0, $CHCl_3$); [lit.[160] $[\alpha]_D^{20}$ = +9.6 (c = 0.2, $CHCl_3$)];IR ($CHCl_3$) *v* 3752, 3155, 2938, 2254, 1816, 1793, 1717, 1643, 1465, 1381, 1096, 905, 717; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.13 (dd, *J* = 5.0, 1.9 Hz, 1H), 4.02 (td, *J* = 8.9, 5.5 Hz, 1H), 1.80-1.58 (m, 2H), 1.47 (s, 6H), 1.54-1.38 (m, 1H), 1.25-1.08 (m, 3H), 0.94 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 107.3, 75.1, 74.6, 39.0, 28.9, 28.0, 25.8, 25.5, 25.3, 21.0, 11.4; HRMS (EI) 184.1463 = $C_{11}H_{20}O_2^+$; calcd. $C_{10}H_{17}O_2^+$ [(M-15)$^+$, CH_3]: 169.1229; found: 169.1232.

(+)-Methyl (5*S*,6*R*)-5,6-Dihydroxycyclohexa-1,3-dienecarboxylate (310)[66]



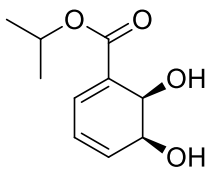
Pale yellow oil; R_f = 0.33 [hexanes/EtOAc (1:2)]; $[\alpha]_D^{20}$ = +68.8 (*c* = 0.8, $CHCl_3$) [lit.[66] $[\alpha]_D^{23}$ = +71.3 (*c* = 1.6, $CHCl_3$)]; IR (film) *v* 3412, 2098, 1690, 1639, 1291, 820, 772; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.05 (d, *J* = 5.3 Hz, 1H), 6.17 (dd, *J* = 10.3, 0.6 Hz, 1H), 6.05 (dq, *J* = 5.1, 2.2 Hz, 1H), 4.50-4.56 (m, 1H), 4.41-4.50 (m, 1H), 3.77 (s, 3H), 3.52-3.67 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.5, 138.6, 134.3, 128.4, 122.6, 69.5, 64.8, 52.1; MS (EI) *m/z* (%) 170 (33), 152 (61), 139 (22), 138 (96), 136 (71), 121 (100), 110 (95), 109 (66), 105 (23), 93 (42), 92 (22) 82 (57), 81 (56), 65 (59), 5 3(49), 51 (22); HRMS (EI) calcd. for $C_8H_{10}O_4$: 170.0579, found: 170.0580.

(+)-*n*-Propyl (5*S*,6*R*)-5,6-Dihydroxycyclohexa-1,3-dienecarboxylate (311)[66]



Off-white waxy solid; R_f = 0.15 [hexanes/EtOAc (1:1)]; $[\alpha]_D^{20}$ = +55.2 (*c* = 1.4, $CHCl_3$) [lit.[66] $[\alpha]_D^{22}$= +58.8 (*c* = 1.1, $CHCl_3$)]; IR (film) *v* 3398, 2968, 1700, 1280, 1240; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.08 (d, *J* = 5.4 Hz, 1H), 6.20 (dd, *J* = 9.5, 2.5 Hz, 1H), 6.09 (ddd, *J* = 9.5, 5.4, 2.2 Hz, 1H), 4.58 (d, *J* = 6.3 Hz, 1H), 4.48 (ddd, *J* = 6.3, 2.5, 2.2 Hz, 1H), 4.16 (t, *J* = 6.7 Hz, 2H), 3.40 (bs, 2H), 1.72 (qt, *J* = 7.4, 6.7 Hz, 2H), 0.98 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (75MHz, $CDCl_3$) δ 167.1, 138.4, 133.9, 128.7, 122.6, 69.4, 66.6, 64.8, 22.0, 10.4; MS (EI) *m/z* (%): 198 (18), 180 (22), 138 (100), 121 (81), 110 (54), 105 (77); HRMS (EI) calcd. for $C_{10}H_{14}O_4$: 198.0892, found: 198.0892.

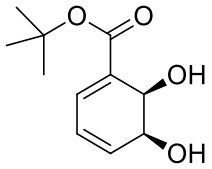
(+)-*i*-Propyl (5*S*,6*R*)-5,6-Dihydroxycyclohexa-1,3-dienecarboxylate (312)[66]



Colourless crystals; R_f = 0.31 [hexanes/EtOAc (2:3)]; mp 80-82 ^{o}C (pentane) [lit.[66] mp 83-85 ^{o}C (EtOAc/hexanes)]; $[\alpha]_D^{20}$ = +66.5 (*c* = 1.0, $CHCl_3$) [lit.[20] $[\alpha]_D^{22}$ = +64.7 (*c* = 1.1, $CHCl_3$)]; IR (film) *v* 3274, 2981, 1698, 1263, 1241; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.05 (ddd, *J* = 5.5, 1.0, 0.5 Hz, 1H), 6.20 (ddt, *J* = 9.6, 2,7, 0.9 Hz, 1H), 6.08 (ddd, *J* = 9.6, 5.5, 2.2 Hz, 1H), 5.12 (hept, *J* = 6.3 Hz, 1H), 4.58 (dd, *J* = 6.4, 0.5 Hz, 1H), 4.48 (br m, 1H),

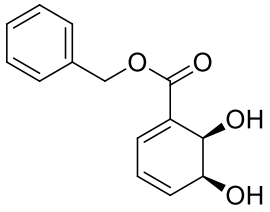
3.60-3.25 (br s, 2H), 1.30 (d, J = 6.3 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.5, 138.2, 133.6, 128.9, 122.63, 69.2, 68.5, 64.9, 21.8; MS (EI) *m/z* (%): 198 (19), 180 (16), 156 (14), 138 (100); HRMS (EI) calcd. for $C_{10}H_{14}O_4$: 198.08921, found: 198.08896; Anal. calcd. for $C_{10}H_{14}O_4$: C, 60.59; H, 7.12. Found C, 60.68; H, 7.19.

(+)-*t*-Butyl (5*S*,6*R*)-5,6-Dihydroxycyclohexa-1,3-dienecarboxylate (313)



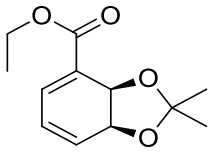
White crystalline solid; R_f = 0.31 [hexanes/EtOAc (1:1)]; mp 88-90 °C (pentane); $[\alpha]_D^{20}$ = +53.3 (c = 0.3, $CHCl_3$); IR (film) v 3382, 2977, 2932, 1699, 1639, 1575, 1477, 1456, 1393, 1368, 1290, 1253, 1159, 1106, 1066, 1024, 993, 889, 849, 826, 783, 770; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.95 (d, J = 5.6 Hz, 1H), 6.22 (dd, J = 9.5, 2.8 Hz, 1H), 6.11 (ddd, J = 9.5, 5.7, 1.9 Hz, 1H), 4.51 (dd, J = 6.4, 4.0 Hz, 1H), 4.50-4.44 (m, 1H), 1.59 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.4, 137.3, 132.9, 129.8, 123.0, 81.6, 68.6, 65.7, 28.1; MS (EI) *m/z* (%): 195 (15), 194 (100), 165 (6), 162 (5), 157 (10), 156 (96), 154 (21), 152 (6); HRMS (EI) 212.1049 = $C_{11}H_{16}O_4$; calcd. for $C_{11}H_{14}O_3$ [$(M-18)^+$, H_2O]: 194.0943, found: 194.0944.

(+)-Benzyl (5*S*,6*R*)-5,6-Dihydroxycyclohexa-1,3-dienecarboxylate (314)



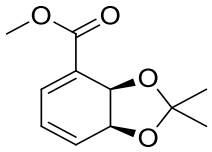
Pale yellow oil; R_f = 0.30 [hexanes/EtOAc (1:1)]; $[\alpha]_D^{20}$ = +48.8 (*c* = 0.2, $CHCl_3$); IR (film) *v* 3390, 2959, 1704, 1639, 1575, 1498, 1455, 1395, 1260, 1164, 1104, 1066, 1027, 995, 915, 811, 757, 697; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.38-7.33 (m, 5H), 7.12 (d, *J* = 5.6 Hz, 1H), 6.23 (dd, *J* = 9.7, 2.8 Hz, 1H), 6.12-6.09 (m, 1H), 5.25 (s, 2H), 4.63-4.61 (m, 1H), 4.50-4.48 (m, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.8, 138.5, 135.7, 134.3, 128.7, 128.4, 128.3, 128.2, 122.8, 68.9, 66.8, 65.2; MS (EI) *m/z* (%): 246 (13), 244 (58), 238 (24), 228 (28), 221 (13), 220 (100), 213 (19), 212 (99), 210 (12), 207 (12), 204 (17), 201 (10), 200 (49), 199 (16), 197 (13), 195 (15), 194 (36), 192 (12), 183 (15), 182 (14), 181 (19), 179 (15), 172 (12), 169 (10), 168 (11), 167 (30), 166 (14), 165 (20), 159 (11), 157 (22), 156 (31), 155 (45), 154 (13), 153 (20), 152 (18), 151 (15); HRMS (EI) 246.0892 = $C_{14}H_{14}O_4$; calcd. for $C_{14}H_{12}O_3$ [$(M-18)^+$, H_2O]: 228.0786, found: 228.0787.

(+)-Ethyl (3a*R*,7a*S*)-2,2-Dimethyl-3a,7a-dihydrobenzo[*d*][1,3]dioxole-4-carboxylate (315)[66]



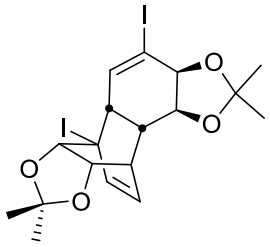
Colourless oil; R_f = 0.56 [hexanes/EtOAc (1:1)]; $[\alpha]_D^{20}$ = +76.2 (*c* = 1.3, $CHCl_3$) [lit.[66] $[\alpha]_D^{23}$ = +74.6 (*c* = 4.0, $CHCl_3$)]; IR (film) *v* 3018, 2987, 2936, 1712, 1651, 1425, 1380, 1259, 1155, 1031, 917, 856, 697, 667, 512; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.16-7.13 (m, 1H), 6.12-6.10 (m, 2H), 4.95 (d, *J* = 8.4 Hz, 1H), 4.87 (dd, *J* = 8.5, 1.7 Hz, 1H), 4.35-4.23 (m, 2H), 1.47 (s, 3H), 1.41 (s, 3H), 1.34 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.2, 142.3, 130.0, 108.5, 72.6, 70.4, 60.5, 27.8, 26.2, 25.1, 20.9, 14.2; MS (EI) *m/z* (%): 211 (77), 181 (15), 169 (17), 123 (100), 105 (17), 95 (13), 83 (11), 79 (76), 67 (14), 59 (10), 55 (11), 43 (82), 41 (14); HRMS (EI) 224.1049 = $C_{12}H_{16}O_4$; calcd. $C_{11}H_{13}O_4$ $[(M-15)^+, CH_3]$: 211.0970, found: 211.0969; Anal. calcd. for $C_{12}H_{16}O_4$: C, 64.27; H, 7.19. Found C, 64.52; H, 7.08.

(+)-Methyl (3a*R*,7a*S*)-2,2-Dimethyl-3a,7a-dihydrobenzo[*d*][1,3]dioxole-4-carboxylate (317) [164(d),184]



Colourless oil; R_f = 0.45 [hexanes/EtOAc (4:1)]; $[\alpha]_D^{20}$ = +88.5 (*c* = 0.2, $CHCl_3$); IR (film) *v* 2988, 2952, 1715, 1651, 1589, 1437, 1371, 1260, 1160, 1108, 1081, 1030, 881, 864, 762, 707, 631, 522; ^{1}H NMR (300 MHz, $CDCl_3$) 7.16-7.13 (m, 1H), 6.12-6.10 (m, 2H), 4.94 (d, *J* = 8.4 Hz, 1H), 4.87 (dd, *J* = 8.5, 1.9 Hz, 1H), 3.83 (s, 3H), 1.46 (s, 3H), 1.40 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.7, 134.0, 133.9, 126.1, 121.3, 105.6, 72.5, 68.2, 52.0, 26.7, 25.1; MS (EI) *m/z* (%) 195 (68), 163 (11), 153 (100), 152 (39), 121 (90), 120 (16), 109 (57), 105 (21), 94 (21), 93 (27), 92 (18), 79 (12), 77 (32), 65 (44), 63 (10), 59 (37), 51 (11); HRMS (EI) 210.0892 = $C_{11}H_{14}O_4$; calcd. $C_{10}H_{11}O_4$ [$(M-15)^+$, CH_3]: 195.0657. Found: 195.0655.

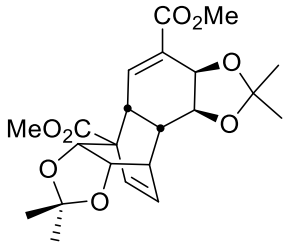
(+)-(3a*S*,5a*S*,6*S*,6a*S*,9a*S*,10*R*,10a*R*,10b*S*)-4,6-Diiodo-2,2,8,8-tetramethyl-3a,5a,6,6a,9a,10,10a,10b-octahydro-6,10-ethenonaphtho[1,2-*d*:6,7-*d'*]bis([1,3]dioxole) (318)



Acetonide **316**[185] was dissolved in *o*-xylene and heated to reflux for 40 hours. The solution was concentrated under reduced pressure and purified by flash column chromatography [hexanes/EtOAc (4:1)] to obtain 0.19 g (38%) of **318** as a white solid.

R_f = 0.39 [hexanes/EtOAc (4:1)]; mp 220-224 °C (EtOAc/pentane); $[\alpha]_D^{20}$ = +106.5 (*c* = 1.0, $CHCl_3$); IR (film) *v* 2984, 2933, 2898, 1455, 1380, 1280, 1262, 1236, 1225, 1209, 1160, 1082, 1068, 1012; ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.89 (d, *J* = 3.7 Hz, 1H), 6.25 (d, *J* = 8.6 Hz, 1H), 5.79 (t, *J* = 7.3 Hz, 1H), 4.49 (d, *J* = 7.2 Hz, 1H), 4.38 (dd, *J* = 7.2, 3.6 Hz, 1H), 4.18 (d, *J* = 4.6 Hz, 1H), 4.15 (d, *J* = 4.1 Hz, 1H), 2.91 (m, 1H), 2.71 (dd, *J* = 8.7, 3.6 Hz, 1H), 2.49 (d, *J* = 9.2 Hz, 1H), 1.47 (s, 3H), 1.40 (s, 3H), 1.38 (s, 3H), 1.36 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 140.1, 139.0, 128.7, 109.3, 108.7, 103.9, 84.7, 79.1, 78.6, 75.2, 48.3, 45.6, 38.4, 35.2, 27.9, 26.7, 25.5, 25.1; MS (EI) *m/z* (%) 122 (11), 107 (14), 105 (11), 104 (43), 93 (11), 91 (26), 79 (23), 78 (10), 77 (23), 75 (27), 70 (12), 69 (11), 61 (14), 57 (18), 55 (15), 45 (19), 43 (100), 42 (11), 41 (18), 39 (15); HRMS (EI) calcd. for $C_{18}H_{22}O_4$: 555.9607, found: 555.9602; Anal. calcd. for $C_{18}H_{22}I_2O_4$: C, 38.87; H, 3.99. Found: C, 39.14; H, 4.04.

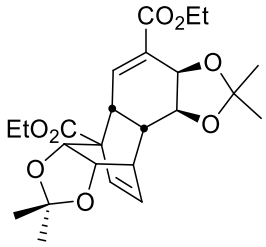
(+)-(3a*R*,5a*R*,6*S*,6a*R*,9a*S*,10*R*,10a*S*,10b*S*)-Dimethyl 2,2,8,8-tetramethyl-3a,5a,6,6a,9a,10,10a,10b-octahydro-6,10-ethenonaphtho[1,2-*d*:6,7-*d'*]bis([1,3]dioxole)-4,6-dicarboxylate (319)



Acetonide **317**[162(d),171] was dissolved in *o*-xylene and heated to reflux for 24 hours. The solution was concentrated under reduced pressure and purified via flash column chromatography [hexanes/EtOAc (6:1)] to obtain 34 mg (42%) of **319** as white powdery solid.

R_f = 0.33 [hexanes/EtOAc (3:1)]; mp 180-183 °C (pentane); $[\alpha]_D^{20}$ = +50.3 (*c* = 0.4, $CHCl_3$); IR (film) *v* 2932, 1737, 1436, 1379, 1283, 1262, 1210, 1164, 1060, 882, 804, 742; ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.45 (d, *J* = 8.3 Hz, 1H), 6.20 (t, *J* = 7.2 Hz, 1H), 5.40 (t, *J* = 2.6 Hz, 1H), 4.63 (dd, *J* = 7.2, 1.4 Hz, 1H), 4.40 (dd, *J* = 7.3, 3.2 Hz, 1H), 4.29 (t, *J* = 7.6 Hz, 1H), 3.90 (s, 3H), 3.79 (s, 3H), 3.63 (t, *J* = 8.2 Hz, 1H), 3.39-3.34 (m, 1H), 3.11 (dt, *J* = 7.4, 2.5 Hz, 1H), 1.94 (dd, *J* = 8.3, 1.9 Hz, 1H), 1.51 (s, 3H), 1.35 (s, 6H), 1.30 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.0, 171.2, 138.8, 129.7, 128.5, 117.8, 110.0, 109.9, 79.0, 78.4, 77.3, 56.3, 52.6, 52.5, 48.9, 42.6, 38.3, 27.2, 25.4, 25.0, 24.6; MS (EI) *m/z* (%) 213 (8), 157 (6), 131 (6), 121 (7), 100 (13), 97 (6), 95 (6), 83 (6), 81 (4), 77 (7), 71 (6), 70 (5), 69 (15), 61 (5), 59 (5), 58 (100), 57 (12), 55 (9); HRMS (EI) calcd. for $C_{22}H_{28}O_8$: 420.1784, found: 420.1801; Anal. calcd. for $C_{22}H_{28}O_8$: C, 62.85; H, 6.71. Found: C, 63.10; H, 6.79.

(+)-(3a*R*,5a*R*,6*S*,6a*R*,9a*S*,10*R*,10a*S*,10b*S*)-Diethyl 2,2,8,8-tetramethyl-3a,5a,6,6a,9a,10,10a,10b-octahydro-6,10-ethenonaphtho[1,2-*d*:6,7-*d'*]bis([1,3]dioxole)-4,6-dicarboxylate (320)

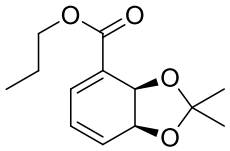


A solution of acetonide **315**[66] was dissolved in *o*-xylene and heated to reflux for 40 hours. The solution was concentrated under reduced pressure and purified via flash column chromatography [hexanes/EtOAc (4:1)] to obtain 0.34 g (68%) of **320** as pale yellow solid.

R_f = 0.24 [hexanes/EtOAc (4:1)]; mp 91-93 °C (pentane); $[\alpha]_D^{20}$ = +47.8 (*c* = 1.0, $CHCl_3$); IR (film) *v* 2984, 2937, 1721, 1458, 1380, 1262, 1221, 1163, 1072, 1026; ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.43 (d, *J* = 3.8 Hz, 1H), 6.38 (d, *J* = 8.8 Hz, 1H), 6.04 (t, *J* = 7.0 Hz, 1H), 4.60 (d, *J* = 7.0 Hz, 1H), 4.58 (d, *J* = 4.8 Hz, 1H), 4.43 (dd, *J* = 7.0, 3.3 Hz, 1H), 4.37 (q, *J* = 13.9 Hz, 2H), 4.18-4.25 (m, 2H), 4.16 (dd, *J* = 4.6, 1.9 Hz, 1H), 3.01 (m, 1H), 2.95 (dd, *J* = 9.0, 3.3 Hz, 1H), 2.32 (d, *J* = 9.1 Hz, 1H), 1.35 (s, 3H), 1.33 (t, *J* = 7.1 Hz, 3H), 1.29 (s, 3H), 1.28 (s, 3H), 1.27 (s, 3H), 1.26 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.5, 165.9, 136.5, 131.5, 130.2, 128.8, 109.7, 108.2, 80.7, 78.3, 76.9, 69.2, 61.5, 60.7, 53.9, 40.4, 38.6, 34.9, 28.1, 26.5, 25.4, 25.1, 14.2, 14.2; MS (EI) *m/z* (%) 433 (22), 390 (27), 375 (22), 287 (22), 286 (25), 285 (65), 273 (14), 272 (27), 269 (53), 259 (20), 258 (17), 257 (26), 245 (17), 244 (22), 241 (32), 229 (26), 228 (14), 227 (100), 217 (14), 213 (27); HRMS (EI) 448.2097 = $C_{24}H_{32}O_8$; calcd. $C_{23}H_{29}O_8$ [$(M-15)^+$, CH_3]:

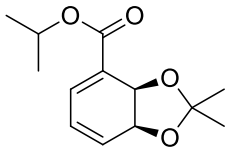
433.1857, found: 433.1857; Anal. calcd. for $C_{24}H_{32}O_8$: C, 64.27; H, 7.19. Found C, 63.99; H, 7.30.

(+)-*n*-Propyl (3a*R*,7a*S*)-2,2-Dimethyl-3a,7a-dihydrobenzo[*d*][1,3]dioxole-4-carboxylate (321)



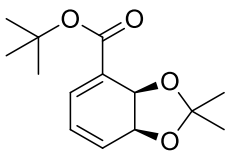
Clear colourless oil; R_f = 0.46 [hexanes/EtOAc (4:1)]; $[\alpha]_D^{20}$ = +72.6 (*c* = 1.2, $CHCl_3$); IR (film) *v* 2974, 2937, 1713, 1589, 1460, 1379, 1370, 1256, 1160, 1107, 1080, 1052, 1027, 863, 705; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.16-7.14 (m, 1H), 6.12-6.10 (m, 2H), 4.95 (d, *J* = 8.3 Hz, 1H), 4.87 (dd, *J* = 8.3, 1.4 Hz, 1H), 4.19 (t, *J* = 6.6 Hz, 2H), 1.73 (sextet, *J* = 14.2, 7.7 Hz, 2H), 1.46 (s, 3H), 1.41 (s, 3H), 1.00 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.2, 133.8, 133.6, 126.4, 121.3, 105.5, 72.6, 68.2, 66.4, 26.7, 25.0, 22.0, 10.5; MS (EI) *m/z* (%) 223 (27), 181 (72), 180 (18), 179 (17), 163 (11), 139 (100), 138 (42), 137 (18), 122 (11), 121 (69), 120 (15), 110 (18), 105 (21), 95 (65), 94 (18), 93 (15), 82 (12), 77 (24), 66 (11), 65 (28), 58 (49); HRMS (EI) 238.1205 = $C_{13}H_{18}O_4$; calcd. $C_{12}H_{15}O_4$ [$(M-15)^+$, CH_3]: 223.0970, found: 223.0968.

(+)-*i*-Propyl (3a*R*,7a*S*)-2,2-Dimethyl-3a,7a-dihydrobenzo[*d*][1,3]dioxole-4-carboxylate (322)



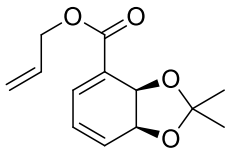
Clear colourless oil; R_f = 0.51 [hexanes/EtOAc (4:1)]; $[\alpha]_D^{20}$ = +94.8 (*c* = 0.1, $CHCl_3$); IR (film) *v* 2983, 2935, 1710, 1589, 1455, 1372, 1261, 1160, 1106, 1027, 927, 862, 803, 706; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.13-7.11 (m, 1H), 6.11-6.09 (m, 2H), 5.16 (heptet, *J* = 18.8, 12.5, 6.2 Hz, 1H), 4.94 (d, *J* = 8.3 Hz, 1H), 4.87 (dd, *J* = 8.3, 1.4 Hz, 1H), 1.47 (s, 3H), 1.41 (s, 3H), 1.31 (d, *J* = 6.3 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.6, 133.7, 133.4, 126.7, 121.3, 105.5, 72.6, 68.2, 68.1, 26.7, 25.0, 21.9, 21.9; MS (EI) *m/z* (%) 223 (19), 181 (68), 180 (19), 179 (19), 139 (100), 138 (55), 137 (20), 122 (14), 121 (96), 120 (22), 110 (17), 105 (18), 95 (49), 94 (21), 93 (19), 92 (12), 82 (18), 77 (24), 66 (15), 65 (35), 59 (16); HRMS (EI) 238.1205 = $C_{13}H_{18}O_4$; calcd. $C_{12}H_{15}O_4$ [$(M-15)^+$, CH_3]: 223.0970, found: 223.0966.

(+)-*t*-Butyl (3a*R*,7a*S*)-2,2-Dimethyl-3a,7a-dihydrobenzo[*d*][1,3]dioxole-4-carboxylate (323)



Clear colourless oil; R_f = 0.63 [hexanes/EtOAc (4:1)]; $[\alpha]_D^{20}$ = +81.3 (*c* = 0.6, $CHCl_3$); IR (film) *v* 2981, 2933, 1707, 1589, 1456, 1368, 1275, 1255, 1160, 1109, 1050, 1025, 905, 848, 704; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.07-7.04 (m, 1H), 6.08-6.07 (m, 2H), 4.87 (d, *J* = 8.2 Hz, 1H), 4.85 (d, *J* = 8.3, 1H), 1.54 (s, 9H), 1.46 (s, 3H), 1.41 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.4, 133.5, 133.1, 127.6, 121.3, 105.5, 81.0, 72.8, 68.2, 28.2, 26.7, 25.0; MS (EI) *m/z* (%) 181 (25), 179 (14), 139 (46), 138 (40), 121 (46), 95 (19), 94 (21), 93 (13), 77 (14), 65 (24), 57 (100); HRMS (EI) 252.1361 = $C_{14}H_{20}O_4$; calcd. $C_{13}H_{17}O_4$ $[(M-15)^+, CH_3]$: 237.1122, found: 237.1127.

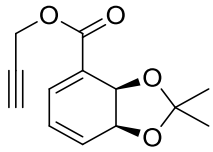
(+)-Allyl (3a*R*,7a*S*)-2,2-Dimethyl-3a,7a-dihydrobenzo[*d*][1,3]dioxole-4-carboxylate (324)



Clear colourless oil; R_f = 0.65 [hexanes/EtOAc (4:1)]; $[\alpha]_D^{20}$ = +79.3 (*c* = 0.2, $CHCl_3$); IR (film) *v* 2987, 2935, 1715, 1649, 1589, 1455, 1404, 1371, 1252, 1161, 1109, 1029, 993, 923, 863, 706; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.19-7.17 (m, 1H), 6.13-6.10 (m, 2H), 6.05-5.92 (m, 1H), 5.38 (dq, *J* = 17.4, 1.7 Hz, 1H), 5.26 (dq, *J* = 10.6, 1.6 Hz, 1H), 4.96 (d, *J* = 8.5 Hz, 1H), 4.88 (dd, *J* = 8.5, 2.1 Hz, 1H), 4.74 (dt, *J* = 5.5, 1.5 Hz, 2H), 1.46 (s, 3H), 1.41 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.8, 134.0, 134.0, 132.2, 126.1, 121.3, 118.0, 105.6, 72.5, 68.1, 65.4, 26.7, 25.0; MS (EI) *m/z* (%) 221 (22), 220 (14), 179 (57), 163 (13), 161 (30), 137 (15), 135 (17), 122 (14), 121 (100), 109 (11), 107 (23), 105 (32),

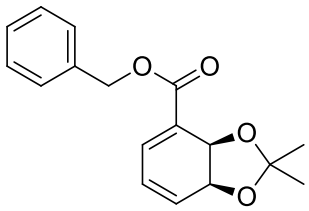
94 (15), 93 (18), 77 (25), 65 (31); HRMS (EI) 236.1049 = $C_{13}H_{16}O_4$; calcd. $C_{12}H_{13}O_4$ $[(M-15)^+, CH_3]$: 221.0814, found: 221.0810.

(+)-Propargyl (3a*R*,7a*S*)-2,2-Dimethyl-3a,7a-dihydrobenzo[*d*][1,3]dioxole-4-carboxylate (325)[66]



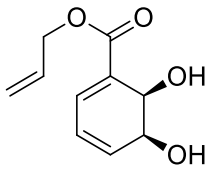
Colourless oil; R_f = 0.54 [hexanes/EtOAc (4:1)]; $[\alpha]_D^{20}$ = +110.6 (*c* = 0.2, $CHCl_3$) [lit.[66] $[\alpha]_D^{20}$ = +112.7 (*c* = 1.3, $CHCl_3$)]; IR (film) *v* 2987, 2935, 1718, 1030; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.22 (dd, *J* = 5.3, 1.1 Hz, 1H), 6.19-6.09 (m, 2H), 4.95 (d, *J* = 8.4 Hz, 1H), 4.89 (dd, *J* = 8.4, 2.4 Hz, 1H), 4.84 (dd, *J* = 2.5, 1.0 Hz, 2H), 2.50 (t, *J* = 2.5 Hz, 1H), 1.47 (s, 3H), 1.41 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.3, 134.8, 134.4, 125.5, 121.2, 105.7, 77.7, 74.9, 71.8, 68.0, 52.3, 26.7, 25.0; MS (EI) *m/z* (%) 219 (42), 177 (41), 163 (17), 121 (83), 43 (100); HRMS (EI) calcd. for $C_{12}H_{11}O_4$: 219.0657, found: 219.0659; Anal. calcd. for $C_{13}H_{14}O_4$: C, 66.66; H, 6.02. Found: C, 66.68; H, 6.08.

(+)-**Benzyl (3a*R*,7a*S*)-2,2-Dimethyl-3a,7a-dihydrobenzo[*d*][1,3]dioxole-4-carboxylate (326)**



Clear colourless oil; R_f = 0.53 [hexanes/EtOAc (4:1)]; $[\alpha]_D^{20}$ = +75.8 (*c* = 1.5, $CHCl_3$); IR (film) *v* 3034, 2987, 2935, 1712, 1588, 1455, 1371, 1253, 1161, 1108, 1028, 864, 737, 698; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44-1.33 (m, 5H), 7.20-7.18 (m, 1H), 6.16-6.07 (m, 2H), 5.28 (s, 2H), 4.98 (d, *J* = 8.4 Hz, 1H), 4.88 (dd, *J* = 8.4, 2.2 Hz, 1H), 1.48 (s, 3H), 1.42 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.0, 136.0, 134.2, 134.1, 128.5, 128.2, 128.1, 126.1, 121.3, 105.6, 72.5, 68.2, 66.5, 26.7, 25.1; MS (EI) *m/z* (%) 105 (15), 91 (100), 77 (14), 65 (25), 58 (11), 52 (15); HRMS (EI) 286.1205 = $C_{17}H_{18}O_4$; calcd. $C_{11}H_{13}O_4$ [$(M-15)^+$, CH_3]: 271.0965, found: 271.0966.

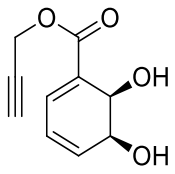
(+)-Allyl (5*S*,6*R*)-5,6-Dihydroxycyclohexa-1,3-dienecarboxylate (327)[66]



Colourless crystals; R_f = 0.23 [hexanes/EtOAc (1:1)]; mp 44-47 ^{o}C (pentane) [lit.[66] mp 48-50 ^{o}C (EtOAc/hexane)]; $[\alpha]_D^{20}$ = +67.4 (*c* = 0.2, $CHCl_3$) [lit.[66] $[\alpha]_D^{22}$ = +72.5 (*c* = 1.6, $CHCl_3$)]; IR (film) *v* 3394, 1704, 1273, 1238; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.12 (d, *J* =

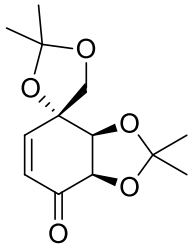
5.5 Hz, 1H), 6.23 (ddt, *J* = 9.6, 2,7, 1.0 Hz, 1H), 6.11 (ddd, *J* = 9.5, 5.5, 2.2 Hz, 1H), 5.98 (ddt, *J* = 17.2, 10.4, 5.7Hz, 1H), 5.37 (dt, *J* = 17.2, 1.5Hz, 1H), 5.28 (dt, *J* = 10.4, 1.3 Hz, 1H), 4.72 (ddd, *J* = 5.7, 1.5, 1.3 Hz, 1H), 4.61 (br s, 1H), 4.50 (br s, 1H), 3.25 (d, *J* = 3.8 Hz, 1H), 3.18 (bd, *J* =7.3Hz, 1H); ^{13}C NMR (75MHz, $CDCl_3$) δ 166.6, 138.8, 134.4, 131.9, 128.4, 122.4, 118.3, 69.6, 65.5, 64.5; MS (EI) *m/z* (%) 196 (20), 178 (18), 138 (80), 121 (95), 41 (100). HRMS (EI) calcd. for $C_{10}H_{12}O_4$: 196.07356, found: 196.07364; Anal. calcd. for $C_{10}H_{12}O_4$ + 1/8 H_2O: C, 60.52; H, 6.22. Found C, 60.52; H, 6.26.

(+)-Propargyl (5*S*,6*R*)-5,6-Dihydroxycyclohexa-1,3-dienecarboxylate (328)[66]



Colourless crystals; R_f = 0.31 [EtOAc/hexanes (3:2)]; mp 67-69 ^{o}C (pentane) [lit.[66] mp 70-72 ^{o}C (EtOAc/Hexanes)]; $[\alpha]_D^{20}$ = +74.3 (*c* = 0.2, $CHCl_3$) [lit.[66] $[\alpha]_D^{22}$ = +88.20 (*c* = 1.6, $CHCl_3$)]; IR (film) *v* 3385, 3291, 1707, 1270, 1234; ^{1}H NMR (300 MHz, acetone-d_6) δ 7.01 (dd, *J* = 5.3, 1.1 Hz, 1H), 6.16 (dq, *J* = 9.5, 1.4 Hz, 1H), 6.09 (ddd, *J* = 9.5, 5.3, 2.2 Hz, 1H), 4.86 (dd, *J* = 15.8, 2.5 Hz, 1H), 4.80 (dd, *J* = 15.8, 2.5 Hz, 1H), 4.50–4.23 (m, 2H), 4.10 (d, *J* = 7.4 Hz, 1H), 3.96 (d, *J* = 5.0 Hz, 1H), 3.06 (t, *J* = 2.5 Hz, 1H); ^{13}C NMR (75 MHz, acetone-d_6) δ 167.2, 142.5, 136.3, 131.1, 123.5, 80.0, 77.3, 72.5, 65.5, 53.4; MS (EI) *m/z* (%) 194 (7), 176 (28), 138 (47), 121 (100); HRMS (EI) calcd for $C_{10}H_{10}O_4$: 194.0579, found: 194.0581; Anal. calcd. for $C_{10}H_{10}O_4$: C, 61.85; H, 5.19. Found: C, 62.08; H, 5.18.

(4*R*,5*S*,6*S*)-4-methyl-6-hydroxy-[5,6;4,4a]-bisdiisopropylidenedioxy-cyclohex-2-en-1-one (331)

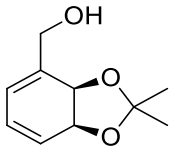


Diol **335** (600 mg, 2.80 mmol) was dissolved in 2,2-dimethoxypropane (10 mL), and to the stirring solution was added a catalytic amount of *p*-TsOH. The reaction was allowed to stir at room temperature until TLC analysis [EtOAc/hexanes (1:4)] indicated the completion of the reaction. The crude reaction mixture was then concentrated under reduced pressure, and re-dissolved in EtOAc (15 mL). The solution was then washed with a saturated aqueous solution of sodium bicarbonate (1 x 3 mL) and brine (1 x 3 mL), dried with MgSO and concentrated under reduced pressure. The crude mixture was then purified by flash column chromatography [EtOAc/hexanes (1:8)] to afford bis-acetonide **331** (555 mg, 2.2 mmol, 78%) as a white powdery solid.

mp 142-144 ºC (ether/pentanes); R_f = 0.56 [EtOAc/hexanes (1:4)]; $[\alpha]_D^{20}$ = +47.1 (c = 1.0, $CHCl_3$); IR ($CHCl_3$) *v* 3691, 3607, 3025, 2992, 2938, 2897, 1689, 1602, 1454, 1384, 1375, 1230, 1064, 852, 765; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.67 (dd, *J* = 10.1, 1.8 Hz, 1H), 6.11 (d, *J* = 10.1 Hz, 1H), 4.45 (d, *J* = 4.8 Hz, 1H), 4.41 (dd, *J* = 4.8, 1.81 Hz, 1H), 4.34 (d, *J* = 9.6 Hz, 1H), 4.03 (d, *J* = 9.6 Hz, 1H), 1.46 (s, 6H), 1.40 (s, 3H), 1.30 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 195.7, 146.4, 128.7, 111.4, 110.6, 79.0, 76.3, 74.5, 71.8, 27.5, 27.3, 26.3, 26.1; MS (EI) *m/z* (%) 239 (40), 179 (20), 154 (58), 139 (38), 121 (37),

100 (65), 96 (100), 85 (68), 68 (47), 43 (70); HRMS (EI) calcd. for $C_{13}H_{18}O_5^+$: 254.1154, found: 254.1147; Anal. calcd. for $C_{13}H_{18}O_5$: C, 61.40; H, 7.14. Found: C, 61.40; H, 7.18.

(5*S*,6*R*)-6-hydroxymethyl-[5,6]-isopropylidenedioxycyclohexa-1,3-diene (333)[186]

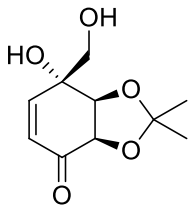


Acetonide **317** (17.4 g (crude), <83mmol) was dissolved in benzene (60 mL) and hexanes (60 mL) and the stirring solution was cooled to 0 ºC. DIBAL-H (neat, 34 mL, 165 mmol) was added dropwise over 5 min. TLC analysis was carried out immediately and indicated that the reaction was complete in <5 min. The reaction mixture was quenched with a saturated aqueous soln. of Rochelle's salt (120 mL) and allowed to stir overnight (12 hours) at room temp. The reaction was then diluted with EtOAc (100 mL), and the aqueous phase extracted with EtOAc (3 x 100 mL). The combined organic phases were washed with sodium bicarbonate (1 x 50 mL) and brine (1 x 50 mL), dried with $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was used directly for the next step.

Clear colourless oil; R_f = 0.61 [EtOAc/hexanes (1:1)]; IR (film) ν 3575, 3416, 3044, 2985, 2930, 1611, 1541, 1406, 1371, 1304, 1246, 1207, 1158, 1033, 950, 877, 820, 791, 756, 718, 694; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.05 (dd, J = 9.4, 5.7 Hz, 1H), 5.98 (ddd, J = 5.6, 2.4, 1.3 Hz, 1H), 5.91 (dd, J = 8.9, 1.1 Hz, 1H), 4.73 (d, J = 1.7 Hz, 2H), 4.35-4.29 (m, 2H), 2.07 (t, J = 5.0 Hz, 1H), 1.43 (s, 3H), 1.43 (s, 3H); ^{13}C NMR (75 MHz,

$CDCl_3$) δ 136.1, 124.7, 124.0, 119.5, 105.5, 71.8, 71.0, 64.6, 26.8, 24.9; MS (EI) *m/z* (%) 153 (10), 139 (16), 123 (16), 121 (23), 105 (17), 95 (12), 85 (13), 83 (15), 77 (11), 73 (20), 71 (36), 70 (13), 69 (16), 59 (32), 57 (100), 56 (40), 55 (41), 45 (23), 44 (33), 43 (97), 42 (53), 41 (59); HRMS (ESI) calcd. for $C_{10}H_{14}O_3Na^+$: 205.0841, found: 205.0834.

(4*R*,5S,6*S*)-4-hydroxymethyl- [5,6]-isopropylidenedioxy-cyclohex-2-en-1-one (335)

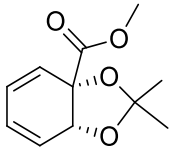


To a solution of endoperoxide **334** (~12.6 g, 59 mmol) in DCM (200 mL) was added triethylamine (0.8 mL, 5.9 mmol). The reaction was allowed to stir for 16 h, when the reaction was observed to be complete by TLC. The crude reaction mixture was concentrated under reduced pressure and purified by flash column chromatography [toluene -> EtOAc/hexanes (1:4)] to provide enone **335** (11.3 g, 53 mmol, 90%) as a white amorphous solid.

White amorphous solid; mp 93-94 ºC (ether); R_f = 0.38 [EtOAc/hexanes (1:8)]; $[\alpha]_D^{20}$ = -25.6º (c = 0.25, $CHCl_3$); IR ($CHCl_3$) *v* 3691, 3603, 3549, 3021, 2938, 1693, 1602, 1376, 1223, 1087, 763; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.79 (dd, *J* = 10.3, 1.0 Hz, 1H), 6.13 (d, *J* = 10.3 Hz, 1H), 4.54-4.46 (m, 2H), 3.80 (d, *J* = 11.5 Hz, 1H), 3.68 (d, *J* = 11.5 Hz, 1H), 1.39 (s, 3H), 1.35 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 195.9, 147.4, 129.2, 110.9, 79.0, 74.5, 70.3, 66.6, 27.2, 25.6; MS (EI) *m/z* (%) 219 (43), 214 (98), 199 (34), 139 (38),

126 (92), 114 (100), 109 (47), 101 (68), 97 (76), 96 (95), 85 (81), 81 (49), 78 (58), 59 (63), 43 (55); HRMS (EI) calcd. for $C_{10}H_{14}O_5^+$: 214.0841, found: 214.0848.

(3a*S*,7a*R*)-methyl 2,2-dimethyl-3a,7a-dihydrobenzo[d][1,3]dioxole-3a-carboxylate[56,93,94(b),187]

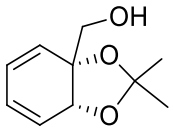


To 75 mL of 40% aqueous solution of KOH was added to 250 mL of diethyl ether, and the stirring solution was cooled to 0 ^{o}C. To the solution was added nitrosomethylurea (25 g, 243 mmol) in portions over 5 minutes. The deep yellow ether layer was then decanted into a solution of acid **4** (7.6 g, 49 mmol) in THF (125 mL) at 0 ^{o}C. The reaction mixture was allowed to warm to room temperature with continuous stirring, and the reaction progress was monitored by TLC. After 4 h the reaction was deemed to be complete, and the ethereal solvents were removed under reduced pressure. The crude mixture was then dissolved in 2,2-DMP (50 mL) and stirred at room temperature. To the mixture was added a catalytic amount of *p*-TsOH, and the reaction progress was monitored by TLC. After 4 h the reaction was deemed to be complete, and the 2,2-DMP was evaporated under reduced pressure. The crude mixture was redissolved in EtOAc (50 mL) and washed with a saturated solution of $NaHCO_3$ (1 x 10mL). The organic solution was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by flash column chromatography [hexanes:EtOAc (4:1)] to afford the title ester as a clear

colourless oil which solidified to a white crystalline solid upon standing (7.4 g, 35 mmol, 70%).

R_f = 0.67 [2:1 (petrol:Et_2O)]; mp 48-51 °C (EtOAc:pentane) [lit.[94(b),187] mp 49-51 °C]; $[\alpha]_D^{20}$ = -397.3 (*c* = 1.0, $CHCl_3$) [lit.[94(b),187] $[\alpha]_D^{20}$ = -417.2 (c = 1.0, $CHCl_3$)]; IR (film) *v* 3041, 2977, 2956, 2924, 1750, 1735, 1453, 1432, 1384, 1368, 1251, 1214, 1166, 1081, 1044, 885, 805, 710; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.10-6.00 (m, 2H), 6.00-5.92 (m, 1H), 5.80-5.73 (m, 1H), 4.90 (d, *J* = 4.1 Hz, 1H), 3.72, (s, 3H), 1.37 (s, 3H), 1.35 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.1, 124.7, 124.5, 124.1, 124.0, 106.8, 79.4, 72.7, 52.9, 26.9, 25.2.

((3aR,7aR)-2,2-dimethyl-3a,7a-dihydrobenzo[d][1,3]dioxol-3a-yl)methanol (336)[56,94(b),187]

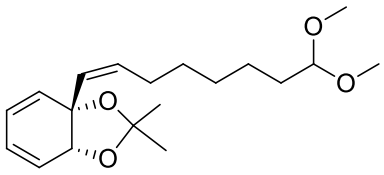


(3a*S*,7a*R*)-methyl 2,2-dimethyl-3a,7a-dihydrobenzo[d][1,3]dioxole-3a-carboxylate (9.8 g, 46.6 mmol) was dissolved in THF (400 mL) and the stirring solution was cooled to 0° C. To the mixture was added a solution of $LiBH_4$ (1.93 g, 88.5 mmol, 2.0 M in THF) dropwise over 5 min. The solution was stirred at 0 ^{o}C for 1 h before being allowed to warm to room temperature over 4 h. The reaction mixture was then quenched with dropwise addition of EtOAc (200 mL) followed by H_2O (300 mL). The resulting

solution was extracted with EtOAc (3 x 300 mL) and the combined organic extracts were dried over $MgSO_4$. The crude extract was concentrated under reduced pressure and purified by flash column chromatography [hexanes:EtOAc (4:1)] to afford alcohol **336** as a clear, colourless oil which solidified to a white crystalline solid upon standing (7.3 g, 40.1 mmol, 86%).

mp 42-44 °C (EtOAc:pentane) [lit. [94(b),187] mp 42-43 °C]; $[\alpha]_D^{20}$ = -211.4 (*c* = 0.8, $CHCl_3$) [lit. [94(b),187] $[\alpha]_D^{20}$ = -215.9 (c = 1.0, $CHCl_3$)]; IR (nujol) ν 3392, 1643, 1594; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.08 (dd, *J* = 9.9, 5.0 Hz, 1H), 5.99 (d, *J* = 9.9 Hz, 1H), 5.98 (d, *J* = 9.9 Hz, 1H), 5.67 (d, *J* = 9.9 Hz, 1H), 4.47 (d, *J* = 5.0 Hz, 1H), 3.56 (d, *J* = 11.5 Hz, 1H), 3.34 (d, *J* = 11.5 Hz, 1H), 2.25 (s, 1H), 1.43 (s, 3H), 1.35 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 128.9, 125.1, 124.4, 123.4, 106.3, 80.5, 70.9, 64.3, 27.1, 26.6.

(3a*S*,7a*R*)-3a-((Z)-8,8-dimethoxyoct-1-en-1-yl)-2,2-dimethyl-3a,7a-dihydrobenzo[d][1,3] dioxole (353)



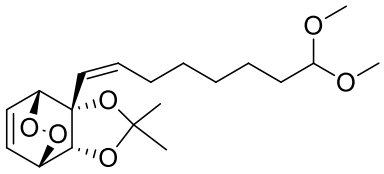
To a stirred solution of DMSO (117 µL, 1.65 mmol), in CH_2Cl_2 (9 mL) at -78 °C was added oxalyl chloride (71 µL, 0.82 mmol), and the solution was allowed to react for 15 min. To the solution was added alcohol **336** (0.10 g, 0.55 mmol) in CH_2Cl_2 (5 mL) dropwise over 5 min, and the solution was allowed to stir for 1 h. Triethylamine (306 µL, 2.20 mmol) was then added at -78 °C, and the solution was allowed to stir for 1 h before

being warmed to room temperature over 12 h. The reaction mixture was then poured into water (14 mL), and the aqueous solution extracted with CH_2Cl_2 (3 × 14 mL). The combined organic layers were washed with brine (1 x 5 mL), dried over $MgSO_4$ and the solvent removed under reduced pressure. The crude residue was passed through a short pad of silica to afford the corresponding aldehyde as a pale yellow oil, which was used immediately without further purification. To a stirring solution of phosphonium bromide **352** (0.33 g, 0.66 mmol) in THF (3 mL) at -78 ^{o}C was added LiHMDS (0.69 mL, 0.9 M in THF) dropwise over 2 min. The reaction mixture was stirred at -78 ^{o}C for 15 min before being allowed to warm to room temperature. The reaction mixture was then cooled to -78 ^{o}C before the dropwise addition of aldehyde (0.10 g, 0.55 mmol) in THF (1 mL) over 5 min. The reaction mixture was stirred at -78 ^{o}C for 3 h before being allowed to warm to room temperature. The reaction was then quenched with a saturated aqueous solution of NH_4Cl (3 mL) and the product extracted with EtOAc (3 x 3 mL). The combined organic extracts were dried over Na_2SO_4, and concentrated under reduced pressure. The crude reaction mixture was purified by flash column chromatography [15:1, hexanes:EtOAc] to afford triene **353** (0.07 g, 0.22 mmol, 40%) as a clear, colourless oil.

R_f = 0.57 [4:1 (hexanes:EtOAc)]; $[\alpha]_D^{20}$ = -195.1 (*c* = 1.9, $CHCl_3$); IR (film) *ν* 2933, 2858, 1456, 1379, 1260, 1210, 1127, 1030, 890, 800, 710; ^{1}H NMR (300 MHz, $CDCl_3$) *δ* 6.11 (dd, *J* = 9.9, 5.5, 1H), 6.01 (dd, *J* = 9.8, 4.5, 1H), 5.86 (ddd, *J* = 9.5, 5.4, 0.9, 1H), 5.76 (d, *J* = 9.5, 1H), 5.58 (dt, *J* = 11.6, 1.4, 1H), 5.45-5.36 (m, 1H), 4.39 (d, *J* = 4.3, 1H), 4.33 (t, *J* = 5.7, 1H), 3.30 (s, 6H), 2.06-1.99 (m, 2H), 1.60-1.53 (m, 2H), 1.41 (s, 3H), 1.37 (s, 3H), 1.34-1.24 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) *δ* 133.3, 133.0, 130.3, 125.1, 124.0,

119.2, 104.8, 104.5, 79.5, 76.2, 52.6, 32.4, 29.5, 29.2, 28.5, 27.0, 25.8, 24.5; MS (EI) m/z (%) 338 (20), 322 (32), 321 (100), 264 (30), 255 (39), 232 (98), 201 (90), 200 (67), 173 (35), 172 (29), 171 (26), 159 (26), 157 (44), 145 (47), 133 (49), 123 (28), 107 (47), 105 (21), 75 (20); HRMS (EI) 322.2144 = $C_{19}H_{30}O_4$; calcd $C_{19}H_{20}O_4$ $[(M-1)^+$, H]: 321.2066, found: 321.2079; Anal. calcd. for $C_{19}H_{30}O_4$: C 70.77, H 9.38; found: C 70.89, H 9.57.

(3a*S*,4*R*,7*S*,7a*R*)-3a-((Z)-8,8-dimethoxyoct-1-en-1-yl)-2,2-dimethyl-3a,4,7,7a-tetrahydro-4,7-epidioxybenzo[d][1,3]dioxole (354)

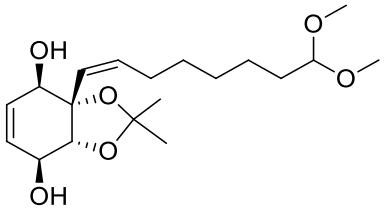


Triene **353** (0.25 g, 0.78 mmol) was dissolved in CH_2Cl_2 (200 mL) in a water-jacketed flask, and tetraphenylporphyrin (0.02 g, 0.04 mmol) was added to the stirring mixture. The solution was irradiated in a water cooled reaction vessel using a 500 W lamp while O_2 was bubbled through continuously. The reaction was monitored using ^{1}H NMR, and the reaction was deemed to be complete after 40 h. The solvent was evaporated under reduced pressure and the crude mixture was purified by column chromatography [toluene to hexanes:EtOAc (9:1)] to afford endoperoxide **354** as a clear colourless oil (0.20 g, 0.56 mmol, 72%).

R_f = 0.32 [4:1 (hexanes:EtOAc)]; $[\alpha]_D^{20}$ = -12.4 (*c* = 1.4, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.65 (td, *J* = 6.2, 1.6 Hz, 1H), 6.50 (td, *J* = 6.2, 1.5 Hz, 1H), 5.73-5.57 (m, 2H), 4.91-4.87 (m, 1H), 4.66 (dt, *J* = 6.1, 1.6 Hz, 1H), 4.37-4.33 (m, 2H), 3.31 (s, 6H), 2.43-

2.26 (m, 2H), 1.63-1.57 (m, 2H), 1.43-1.33 (m, 6H), 1.31 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 135.4, 132.4, 132.0, 129.4, 110.9, 104.5, 79.7, 78.0, 77.8, 72.6, 52.6, 32.4, 29.5, 29.2, 28.7, 26.9, 26.2, 24.5.

(3a*S*,4*R*,7*S*,7a*R*)-3a-((*Z*)-8,8-dimethoxyoct-1-en-1-yl)-2,2-dimethyl-3a,4,7,7a-tetrahydrobenzo[d][1,3]dioxole-4,7-diol (356)

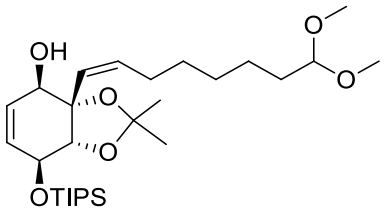


A solution of thiourea (5 mg, 0.06 mmol) in methanol (0.5 mL) was added dropwise over 20 min to a stirred solution of endoperoxide **354** (20 mg, 0.05 mmol) in CH_2Cl_2 (1 mL) at room temperature. The reaction was monitored by TLC and after 1.5 h was deemed to be complete. The crude reaction mixture was then filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography [hexanes:EtOAc (4:1)] to afford diol **356** as a clear colourless oil (15 mg, 0.04 mmol, 74%).

R_f = 0.41 [1:1 (hexanes:EtOAc)]; $[\alpha]_D^{20}$ = +8.7 (*c* = 0.9, $CHCl_3$); IR (film) ν 3433, 2987, 2933, 2856, 1455, 1380, 1259, 1212, 1127, 1056, 885, 789, 718; ^{1}H NMR (300 MHz, MeOD) δ 5.75 (s, 2H), 5.54 (dt, *J* = 12.0, 7.5, 1H), 5.27 (dt, *J* = 12.0, 1.5 Hz, 1H), 4.37 (t, *J* = 5.7 Hz, 1H), 4.27-4.25 (m, 1H), 4.14-4.13 (m, 1H), 4.09 (d, *J* = 3.1 Hz, 1H), 3.33 (s, 6H), 2.51-2.36 (m, 2H), 1.63-1.56 (m, 2H), 1.48 (s, 3H), 1.43 (s, 3H), 1.41-1.36 (m, 6H);

^{13}C NMR (75 MHz, MeOD) δ 136.3, 132.4, 131.5, 129.2, 109.4, 104.5, 85.6, 85.2, 71.2, 68.4, 52.7, 52.6, 32.4, 29.6, 29.2, 28.6, 27.5, 25.9, 24.2; MS (EI) m/z 341 (17), 324 (16), 294 (14), 293 (100), 270 (88), 239 (52), 238 (98), 235 (36), 181 (35), 149 (43), 148 (28), 125 (25), 120 (39), 107 (30), 99 (20), 75 (67); HRMS (EI) 356.2199 = $C_{19}H_{32}O_6$; calcd $C_{19}H_{32}O_6$ [$(M-15)^+$, CH_3]: 341.1985, found: 341.1964.

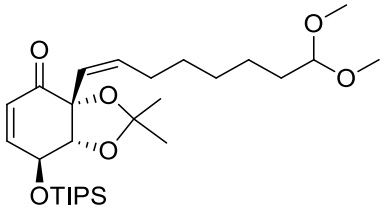
(3aS,4R,7S,7aR)-3a-((Z)-8,8-dimethoxyoct-1-en-1-yl)-2,2-dimethyl-7-((triisopropylsilyl)oxy)-3a,4,7,7a-tetrahydrobenzo[d][1,3]dioxol-4-ol (357)



To a stirring solution of diol **356** (0.35 mg, 0.98 mmol) and 2,6-lutidine (0.25 mL, 2.16 mmol) in CH_2Cl_2 (15 mL) at -78°C was added triisopropylsilane triflate (0.29 mL, 1.08 mmol) dropwise over 5 min. The reaction mixture was slowly warmed up to room temperature over 3 h. A saturated aqueous solution of NH_4Cl (20 mL) was added and the aqueous layer extracted with CH_2Cl_2 (3 x 20 mL). The combined organic extracts were dried with Mg_2SO_4, filtered and concentrated under reduced pressure. The crude mixture was purified by flash column chromatography [4:1 (hexanes: EtOAc)] to afford the silyl ether **357** (0.33 mg, 0.64 mmol, 63%) as a clear colourless oil.

R_f = 0.37 [4:1 (hexanes:EtOAc)]; $[\alpha]_D^{20}$ = +25.4 (*c* = 1.0, $CHCl_3$); IR (film) *v* 3469, 2942, 2866, 1463, 1380, 1258, 1212, 1125, 1060, 883, 683; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.79-5.70 (m, 2H), 5.52 (dt, *J* = 12.0, 7.5 Hz, 1H), 5.31 (dt, *J* = 12.0, 1.5 Hz, 1H), 4.47-4.45 (m, 1H), 4.37 (t, *J* = 5.7 Hz, 1H), 4.15 (d, *J* = 2.7 Hz, 1H), 4.14-4.12 (m, 1H), 3.32 (s, 6H), 2.41 (m, 2H), 1.62-1.56 (m, 2H), 1.47 (s, 3H), 1.41 (s, 3H), 1.40-1.37 (m, 6H), 1.16-1.10 (m, 22H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 134.4, 133.9, 130.8, 129.8, 108.9, 104.5, 85.6, 85.3, 70.7, 68.4, 52.6, 52.5, 32.4, 29.8, 29.3, 28.6, 27.5, 26.0, 24.6, 17.9, 12.0; MS (EI) m/z (%) 462 (24), 450 (21), 449 (86), 448 (79), 438 (20), 437 (100), 405 (15), 404 (14), 391 (28), 390 (19), 380 (16), 379 (62), 373 (15), 372 (21), 362 (14), 361 (43), 345 (20); HRMS (EI) 512.3533 = $C_{28}H_{52}O_6Si$; calcd $C_{27}H_{48}O_5Si$ [$(M-32)^+$, CH_3OH]: 480.3271. Found: 480.4282.

(3a*R*,7*S*,7a*R*)-3a-((Z)-8,8-dimethoxyoct-1-en-1-yl)-2,2-dimethyl-7-((triisopropylsilyl)oxy)-7,7a-dihydrobenzo[d][1,3]dioxol-4(3aH)-one (359)

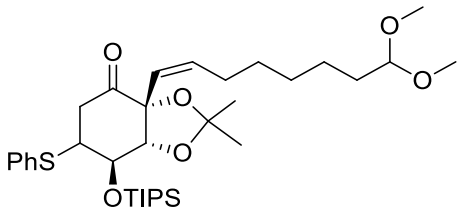


Silyl ether **357** (215 mg, 0.42 mmol) was dissolved in ethyl acetate (20 mL) and IBX (123 mg, 0.46 mmol) was added in portions. The reaction mixture was heated to reflux and monitored by TLC and the reaction was observed to be complete after 4 h. The crude suspension was filtered, washed with a saturated aqueous solution of $NaHCO_3$ (1 x 5 mL), and the organic solution was concentrated under reduced pressure. The crude

reaction mixture was purified by flash column chromatography [4:1 (hexanes:EtOAc)] to afford enone **359** (196 mg, 0.38 mmol, 91%) as a pale yellow oil.

R_f = 0.43 [4:1 (hexanes:EtOAc)]; $[\alpha]_D^{20}$ = -69.5 (*c* = 1.6, $CHCl_3$); IR (film) *v* 2942, 2867, 1698, 1463, 1383, 1229, 1127, 1077, 1053, 882, 844, 790, 684; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.74 (ddd, *J* = 10.2, 4.5, 1.8 Hz, 1H), 6.06 (d, *J* = 10.2 Hz, 1H), 5.59 (dt, *J* = 11.6, 7.4 Hz, 1H), 5.44 (d, *J* = 11.7 Hz, 1H), 4.70 (d, *J* = 4.5 Hz, 1.0, 1H), 4.34 (t, *J* = 5.7 Hz, 1H), 4.25 (t, *J* = 5.7 Hz, 1H), 3.30 (s, 6H), 2.55-2.39 (m, 1H), 2.39-2.22 (m, 1H), 1.63-1.51 (m, 2H), 1.44-1.26 (m, 12H), 1.25-0.99 (m, 22H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 196.8, 144.4, 137.3, 127.7, 122.7, 109.3, 104.6, 83.3, 83.0, 65.4, 52.6, 32.5, 29.5, 29.1, 28.7, 27.4, 26.5, 24.6, 17.9, 12.2; MS (EI) m/z (%) 510 (20), 502 (97), 479 (68), 429 (63), 421 (38), 414 (100), 403 (47), 223 (69), 205 (41), 174 (66), 162 (92), 146 (98), 131 (82), 103 (74), 91 (50); HRMS (EI) $C_{28}H_{50}O_6Si$ calcd: 510.3377, found: 510.3362.

(3a*R*,7*R*,7a*R*)-3a-((Z)-8,8-dimethoxyoct-1-en-1-yl)-2,2-dimethyl-6-(phenylthio)-7-((triisopropylsilyl)oxy)tetrahydrobenzo[d][1,3]dioxol-4(3aH)-one (363)

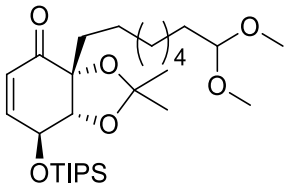


To a stirring solution of enone **359** (70 mg, 0.14 mmol) in CH_2Cl_2 (3 mL) was added thiophenol (15 µL, 0.15 mmol) and Et_3N (3 µL, 0.014 mmol) at room temperature, and the reaction was monitored by TLC. After 12 h the reaction was deemed to be complete, and the reaction mixture concentrated under reduced pressure. The crude mixture was

purified by flash column chromatography [9:1 (hexanes:EtOAc)] to afford the corresponding thioadduct **363** (74 mg, 0.12 mmol, 87%) as a clear, colourless oil.

R_f = 0.55 [4:1 (hexanes:EtOAc)]; $[\alpha]_D^{20}$ = -81.5 (*c* = 1.7, $CHCl_3$); IR (film) *v* 2946, 2867, 1738, 1463, 1382, 1245, 1226, 1126, 882, 800, 692; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46-7.43 (m, 2H), 7.34-7.23 (m, 3H), 5.65-5.59 (m, 2H), 4.64 (s, 1H), 4.34 (t, *J* = 5.7 Hz, 1H), 4.28 (t, *J* = 1.8 Hz, 1H), 3.70-3.66 (m, 1H), 3.30 (s, 6H), 3.23 (dd, *J* = 13.9, 4.7 Hz, 1H), 2.44 (dd, *J* = 13.9, 2.7 Hz, 1H), 2.44-2.37 (m, 1H), 2.26-2.15 (m, 1H), 1.60-1.54 (m, 5H), 1.38 (s, 3H), 1.34-1.28 (m, 6H), 1.18-1.04 (m, 21H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 204.7, 138.8, 135.1, 133.4, 129.2, 127.9, 122.9, 110.4, 104.6, 86.4, 86.3, 70.9, 52.9, 52.6, 39.4, 32.4, 29.3, 29.1, 28.5, 27.0, 26.6, 24.5, 18.0, 12.2; HRMS (EI) 620.3567 = $C_{34}H_{56}O_6SSi$; calcd $C_{34}H_{56}O_6SSiNa$ [$(M+23)^+$, Na]: 643.3459. Found: 643.3414.

(3a*R*,7*S*,7a*R*)-3a-(8,8-dimethoxyoctyl)-2,2-dimethyl-7-((triisopropylsilyl)oxy)-7,7a-dihydrobenzo[d][1,3]dioxol-4(3aH)-one (**364**)

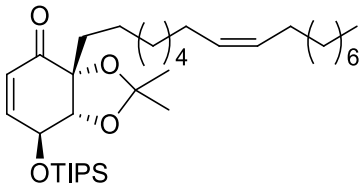


Thioadduct **363** (74 mg, 0.12 mmol) was dissolved in MeOH (2 mL), and Wilkinson's catalyst (130 mg, 0.14 mmol), was added to the methanolic solution. The reaction mixture was allowed to stir at room temperature under 1 atm of H_2. The reaction progress was monitored by TLC, and after 20 h the reaction was deemed to be complete. The crude mixture was filtered through a short pad of silica to afford the corresponding

thioether [^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46-7.42 (m, 2H), 7.35-7.27 (m, 3H), 4.71-4.67 (m, 1H), 4.33 (t, J = 5.7 Hz, 1H), 4.24 (t, J = 1.9 Hz, 1H), 3.68-3.65 (m, 1H), 3.30 (s, 6H), 3.20 (dd, J = 14.0, 4.7 Hz, 1H), 2.51 (ddd, J = 14.0, 2.0, 1.0 Hz, 1H), 1.93-1.84 (m, 1H), 1.80-1.69 (m, 1H), 1.60-1.53 (m, 2H), 1.38 (s, 3H), 1.29-1.16 (m, 13H), 1.14-1.07 (m, 21H)], which was then dissolved in CH_2Cl_2 (2 mL) and stirred at room temperature. To the solution was added DBU (21 μL, 0.15 mmol), and the reaction progress was monitored by TLC. The reaction was deemed to be complete after 8 h. The crude reaction mixture was concentrated under reduced pressure and purified by flash column chromatography to afford enone **364** as a clear colourless oil (37 mg, 0.07 mmol, 61%).

R_f = 0.58 [4:1 (hexanes:EtOAc)]; $[\alpha]_D^{20}$ = +44.6 (c = 0.8, $CHCl_3$); IR (film) ν 2943, 2867, 1694, 1464, 1382, 1240, 1127, 1065, 882, 844, 682; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.77 (ddd, J = 10.2, 4.7, 1.9, 1H), 6.06 (d, J = 10.2, 1H), 4.72 (dd, J = 4.9, 1.6, 1H), 4.35 (t, J = 5.7, 1H), 4.26 (t, J = 1.6, 1H), 3.32 (s, 6H), 1.82-1.76 (m, 2H), 1.59-1.54 (m, 2H), 1.41 (s, 3H), 1.34-1.24 (m, 13H), 1.15-1.08 (m, 21H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 200.0, 144.3, 127.5, 108.2, 104.6, 82.4, 80.9, 65.5, 52.6, 33.8, 32.5, 29.8, 27.3, 26.5, 24.6, 23.2, 18.0, 17.9, 12.2.

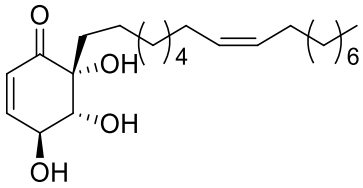
(3a*R*,7*S*,7aR)-3a-((Z)-heptadec-8-en-1-yl)-2,2-dimethyl-7-((triisopropylsilyl)oxy)-7,7a-dihydrobenzo[d][1,3]dioxol-4(3aH)-one (**367**)



Enone **364** (35 mg, 0.07 mmol) was dissolved in prepared solution of iodine (1.7 mg, 0.007 mmol) in acetone (0.6 mL), stirred at room temperature, and the reaction progress was monitored by TLC. After 10 min the reaction was deemed to be complete, the reaction mixture was diluted with CH_2Cl_2 (1 mL) and quenched with 5% aqueous solution of $Na_2S_2O_3$ (0.5 mL). The aqueous layer was separated and extracted with CH_2Cl_2 (3 x 0.5 mL). The combined organic extracts were washed with brine (1 x 0.3 mL), dried with Na_2SO_4 and the solvent was removed under reduced pressure to afford aldehyde **365** [^{1}H NMR (300 MHz, $CDCl_3$) δ 9.75 (t, J = 1.8 Hz, 1H), 6.76 (ddd, J = 10.2, 4.7, 1.8 Hz, 1H), 6.05 (d, J = 10.1 Hz, 1H), 4.71 (dd, J = 4.6, 1.5 Hz, 1H), 4.24 (t, J = 1.6 Hz, 1H), 2.40 (td, J = 7.3, 1.7 Hz, 2H), 1.80-1.75 (m, 2H), 1.62-1.51 (m, 2H), 1.39 (s, 3H), 1.33-1.26 (m, 11H), 1.14-1.06 (m, 21H)], which was used immediately in the next step. To a stirring solution of phosphonium bromide **366** (35 mg, 0.08 mmol) in THF (1 mL) at -78 ^{o}C was added *n*-BuLi (38 µL, 2.11 M) dropwise. The reaction mixture was maintained at -78 ^{o}C before warming to room temperature. The reaction mixture was then cooled to -78 ^{o}C before the dropwise addition of aldehyde **365** over 5 min. The reaction mixture was maintained at -78 ^{o}C for 3 h before being allowed to warm to room temperature. The reaction was then quenched with a saturated aqueous solution of NH_4Cl (1 mL) and the product extracted with EtOAc (3 x 1 mL). The combined organic extracts were dried over Na_2SO_4, and concentrated under reduced pressure. The crude reaction mixture was purified by flash column chromatography [15:1, hexanes:EtOAc] to afford olefin **367** (22 mg, 0.04 mmol, 57%) as a clear, colourless oil.

R_f = 0.53 [9:1 (hexanes:EtOAc)]; $[\alpha]_D^{20}$ = +36.0 (*c* = 0.4, $CHCl_3$); IR (film) *v* 2926, 2855, 1695, 1463, 1372, 1240, 1177, 1065, 882, 845, 683; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.75 (ddd, *J* = 10.2, 4.7, 1.9, 1H), 6.04 (d, *J* = 10.2, 1H), 5.38-5.28 (m, 2H), 4.71 (dd, *J* = 4.8, 1.4, 1H), 4.25 (t, *J* = 1.6, 1H), 2.03-1.97 (m, 4H), 1.83-1.73 (m, 2H), 1.39 (s, 3H), 1.32-1.21 (m, 24H), 1.18-1.07 (m, 22H), 0.88 (t, *J* = 7.0, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 200.0, 144.3, 129.9, 129.8, 127.5, 108.3, 82.5, 80.9, 65.5, 33.8, 31.9, 29.9, 29.8, 29.7, 29.5, 29.4, 29.3, 27.3, 27.2, 27.2, 26.5, 23.2, 22.7, 17.9, 14.1, 12.2; MS (EI) m/z 576 (16), 475 (73), 447 (18), 336 (100), 321 (12), 241 (14), 240 (83), 157 (15); HRMS (EI) $C_{35}H_{64}O_4Si$ calcd: 576.4574, found: 576.4568.

Pleiogenone A (**14**)[100]

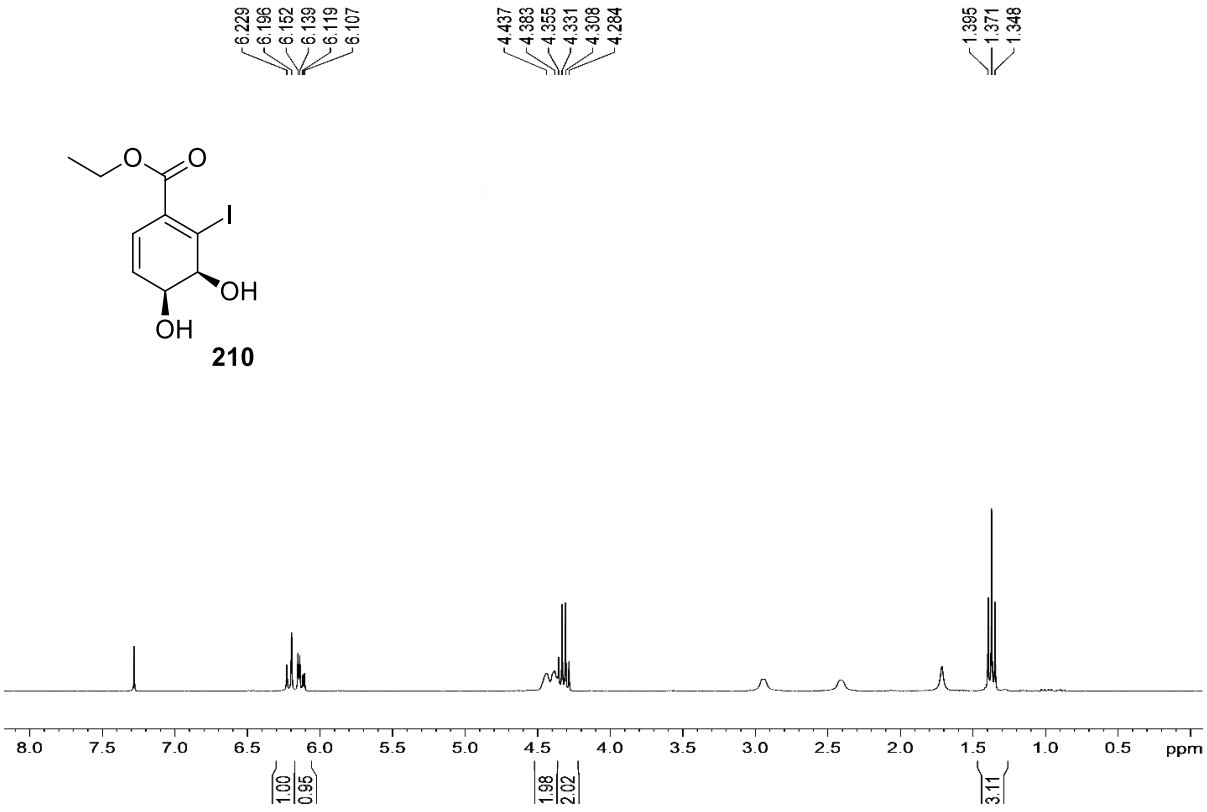


Enone **367** (5.0 mg, 0.009 mmol) was dissolved in a prepared solution of iodine (0.75 mg, 0.003 mmol) in acetonitrile (0.2 mL), and the solution was stirred at room temperature. The reaction progress was monitored by TLC, and the reaction was deemed to be complete after 10 h. The stirring mixture was diluted with CH_2Cl_2 (1 mL) and quenched with 5% aqueous solution of $Na_2S_2O_3$ (0.5 mL). The aqueous layer was separated and extracted with CH_2Cl_2 (3 x 0.5 mL), and the combined organic extracts were washed with brine (1 x 0.3 mL) and dried with Na_2SO_4. The solvent was removed under reduced pressure and the crude reaction mixture was purified by flash column

chromatography [4:1 (hexanes:EtOAc)] to afford pleiogenone A (**14**, 2.1 mg, 0.006 mmol, 64%) as a clear, colourless oil.

$[\alpha]_D^{20}$ = +22.1 (*c* = 0.2, $CHCl_3$) [lit.[100] $[\alpha]_D^{22}$ = +23 (c = 0.5, $CHCl_3$)]; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.80 (ddd, *J* = 10.1, 3.9, 1.4, 1H), 6.10 (dd, *J* = 10.1, 0.8 Hz, 1H), 5.34 (m, 2H), 4.62 (brs, 1H), 3.98 (brs, 1H), 2.00 (m, 4H), 1.83 (m, 2H), 1.26 (m, 20H), 1.13 (m, 2H), 0.88 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 200.2, 145.8, 129.9, 129.8, 126.4, 78.1, 75.4, 68.6, 38.9, 31.6, 29.8, 29.8, 29.7, 29.5, 29.3, 29.3, 29.3, 29.2, 27.2, 27.2, 23.0, 22.7, 14.1; HRMS (ESI) 380.29 = $C_{23}H_{40}O_4$; calcd $C_{23}H_{41}O_4$ [$(M+1)^+$ H]: 381.2999. Found: 381.2972.

6. Selected Spectra



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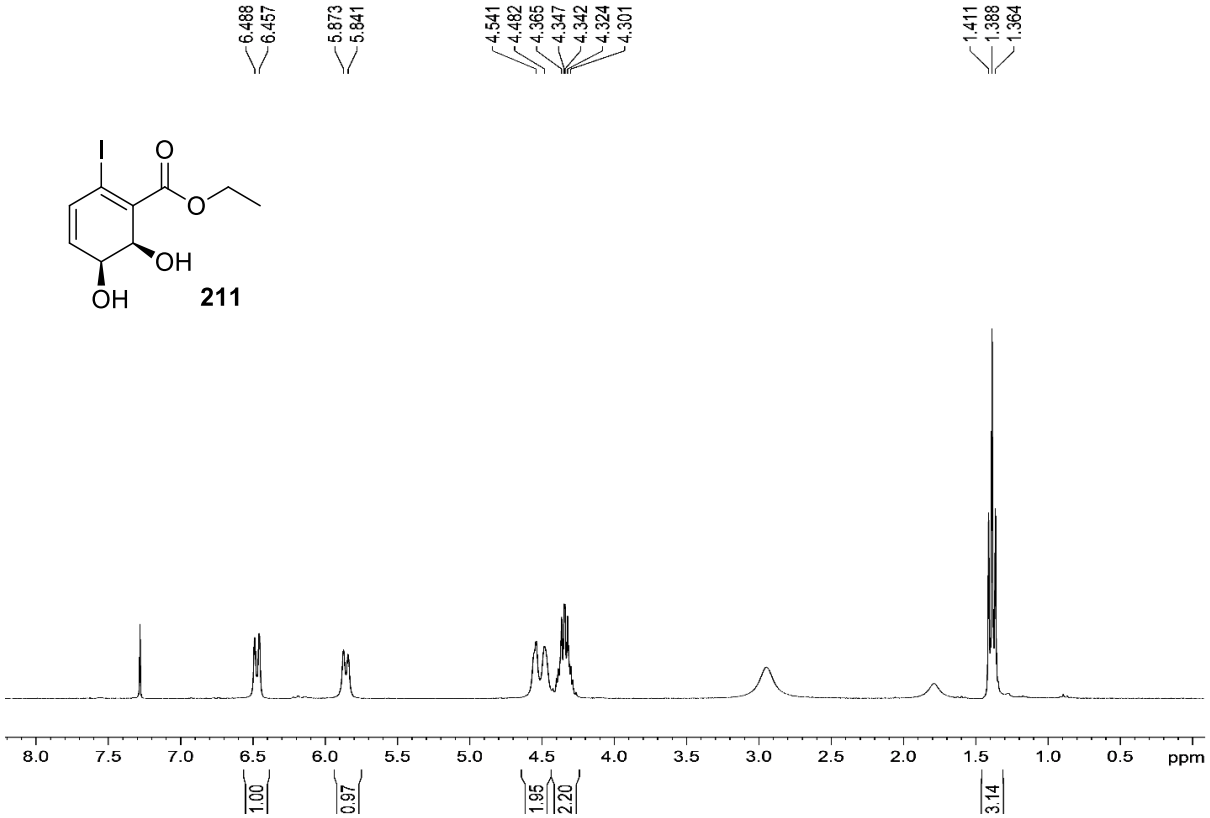
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3.14


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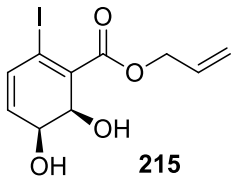
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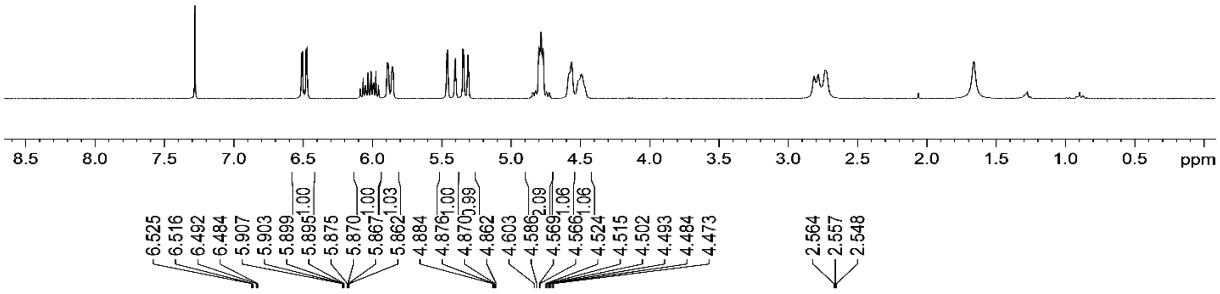
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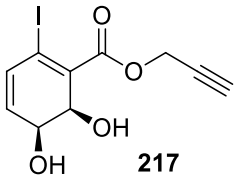
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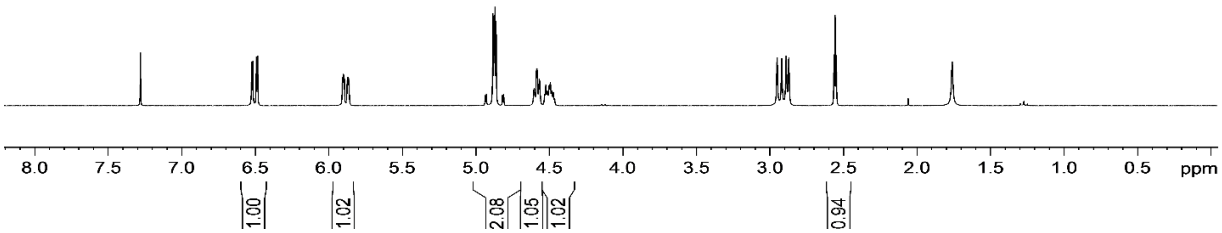
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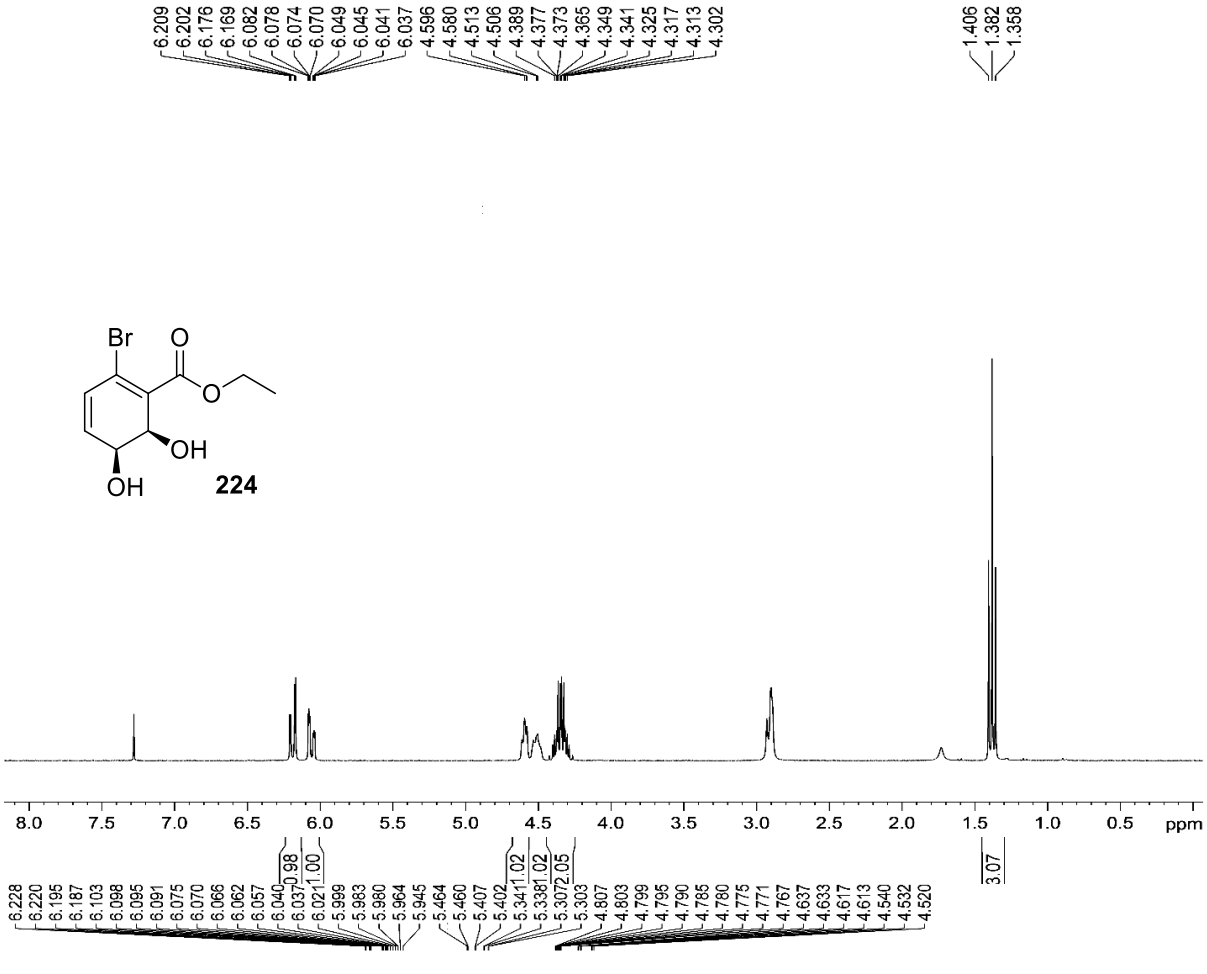
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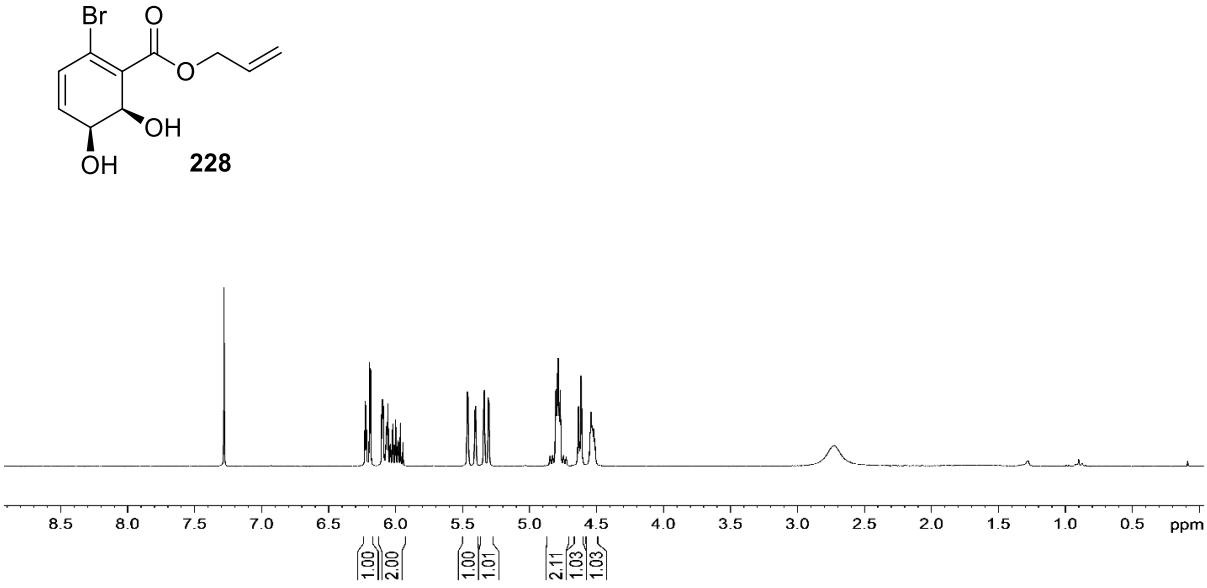
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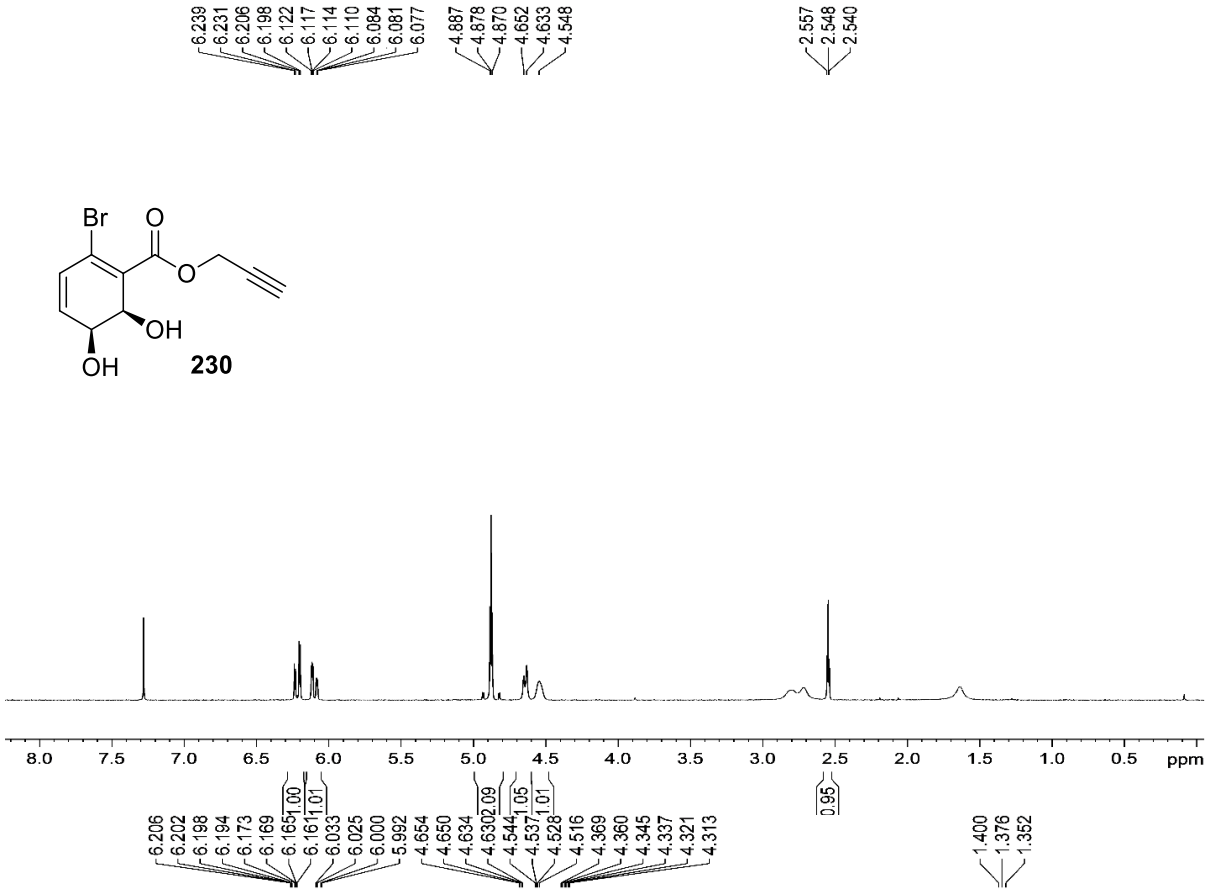
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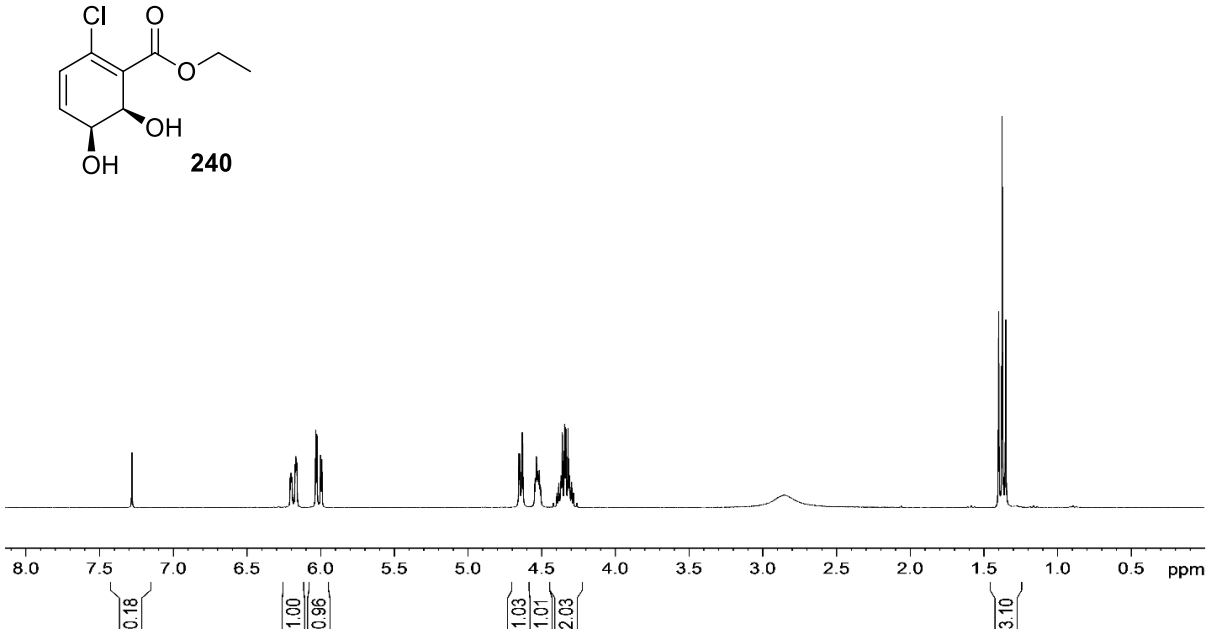
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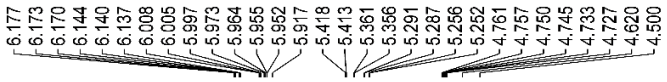
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5.361
5.356
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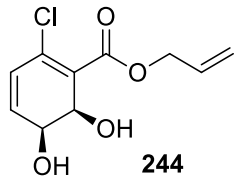
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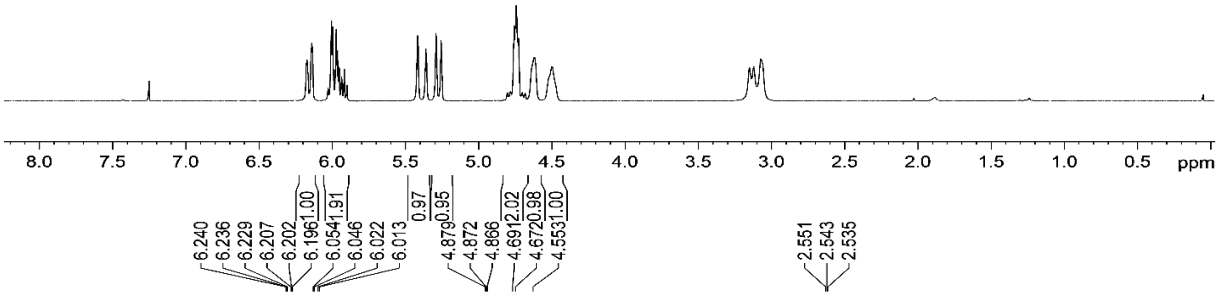
O
OH
OH

244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1.0
0.5
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0.97
0.95
2.02
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1.00
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6.236
6.229
6.207
6.202
6.196
6.054
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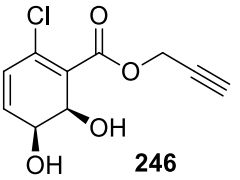
2.535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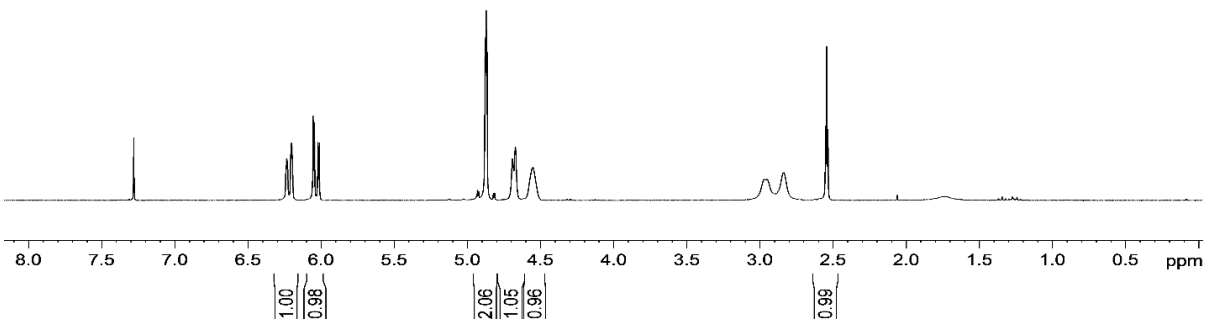
O
O
OH
OH

246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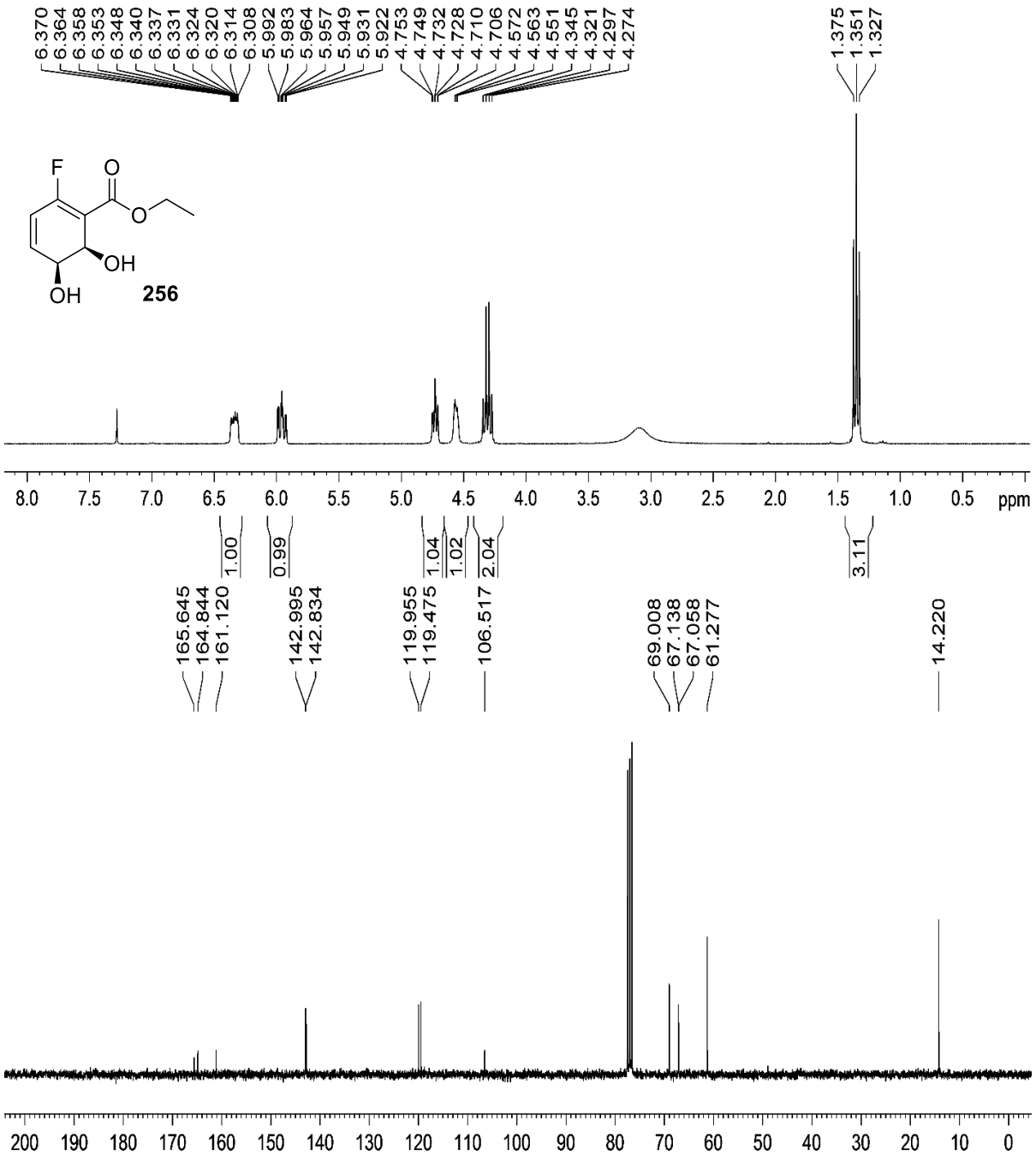
0.99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6.331
6.324
6.320
6.314
6.308
5.992
5.983
5.964
5.957
5.949
5.931
5.922
4.753
4.749
4.732
4.728
4.710
4.706
4.572
4.563
4.551
4.345
4.321
4.297
4.274
1.375
1.351
1.327
F
O
O
OH
OH
256
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7.0
6.5
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5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
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0.99
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1.02
2.04
3.11
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164.844
161.120
142.995
142.834
119.955
119.475
106.517
69.008
67.138
67.058
61.277
14.220
200
190
180
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150
140
130
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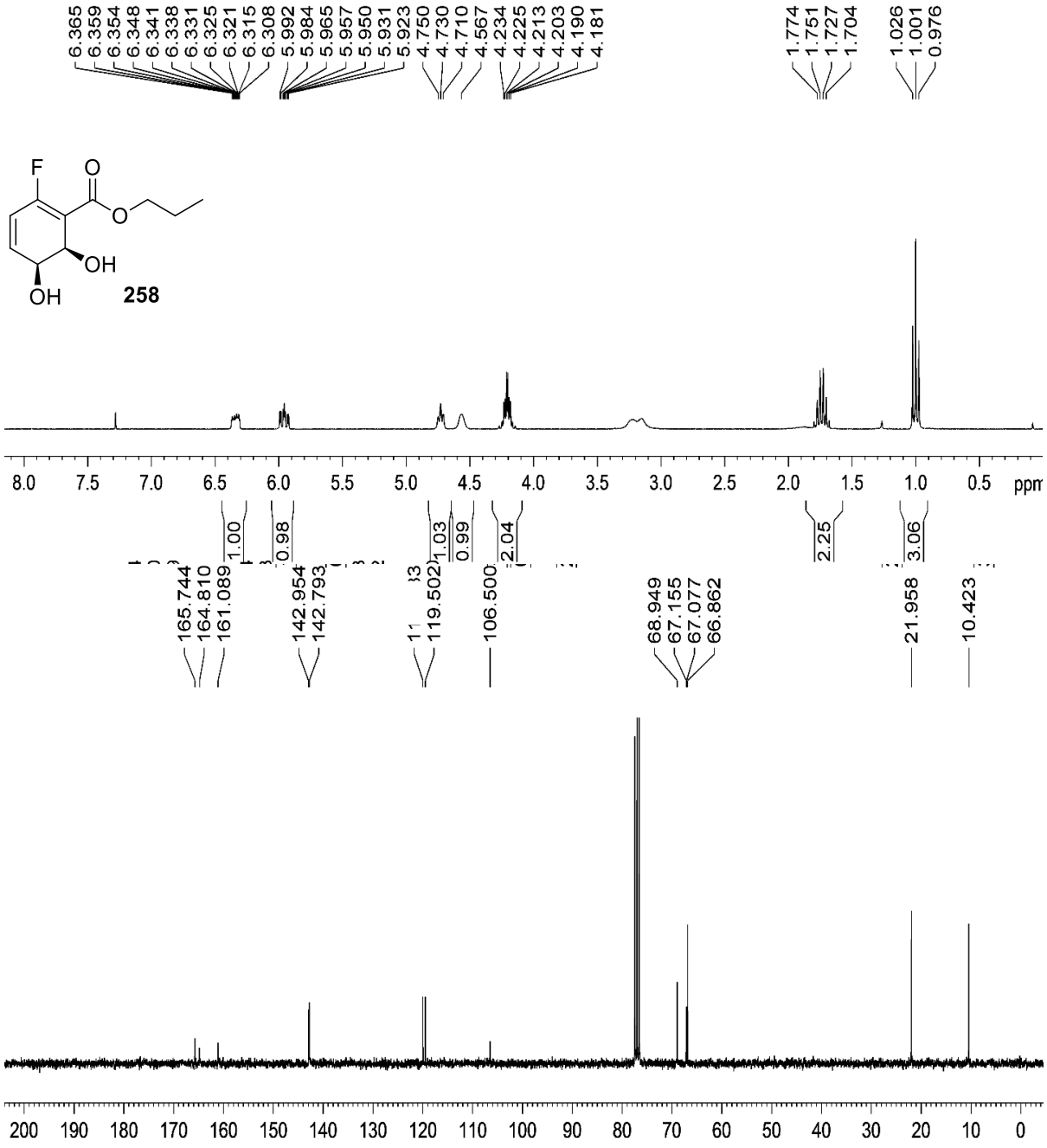
70
60
50
40
30
20
10
0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5.957
5.950
5.931
5.923
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4.730
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4.567
4.234
4.225
4.213
4.203
4.190
4.181
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1.751
1.727
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1.026
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0.976
F
O
O
OH
OH
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5.0
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2.5
2.0
1.5
1.0
0.5
ppm
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1.03
0.99
2.04
2.25
3.06
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164.810
161.089
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142.793
119.502
106.500
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67.077
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21.958
10.423
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10
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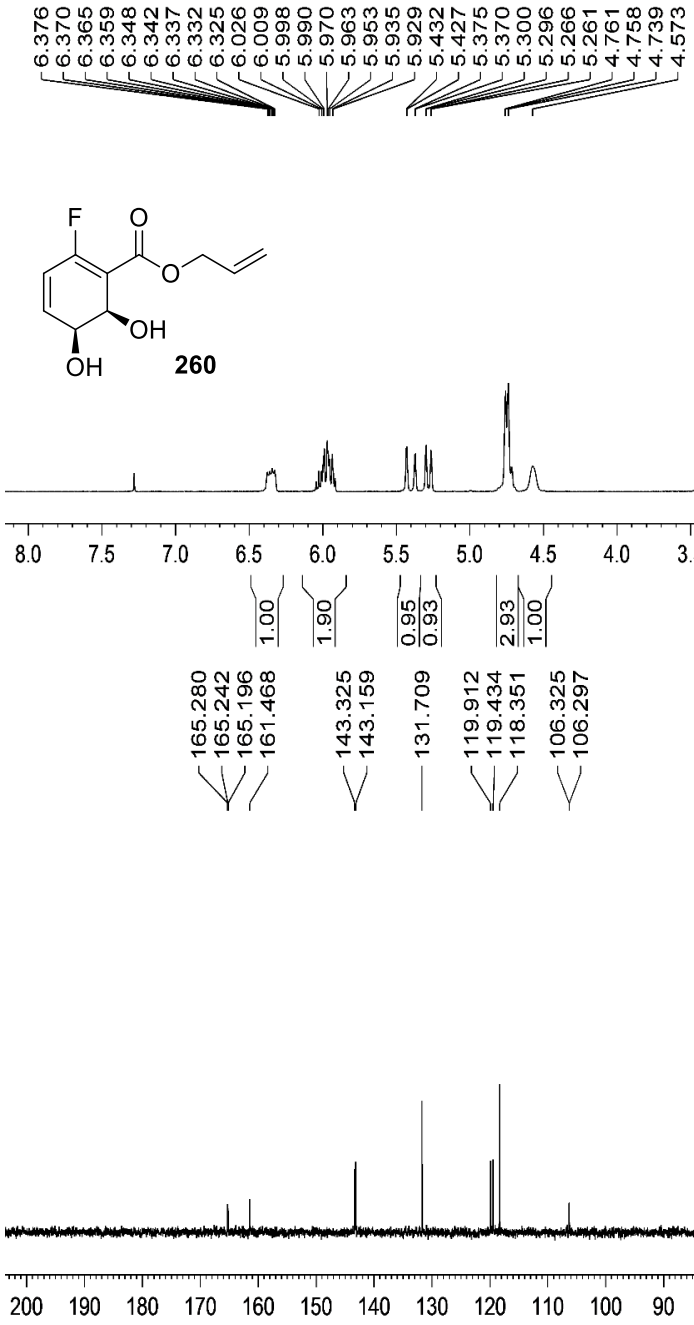
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6.359
6.348
6.342
6.337
6.332
6.325
6.026
6.009
5.998
5.990
5.970
5.963
5.953
5.935
5.929
5.432
5.427
5.375
5.370
5.300
5.296
5.266
5.261
4.761
4.758
4.739
4.573
F
O
O
OH
OH
260
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
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0.95
0.93
2.93
1.00
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165.242
165.196
161.468
143.325
143.159
131.709
119.912
119.434
118.351
106.325
106.297
69.069
67.075
66.993
65.726
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150
140
130
120
110
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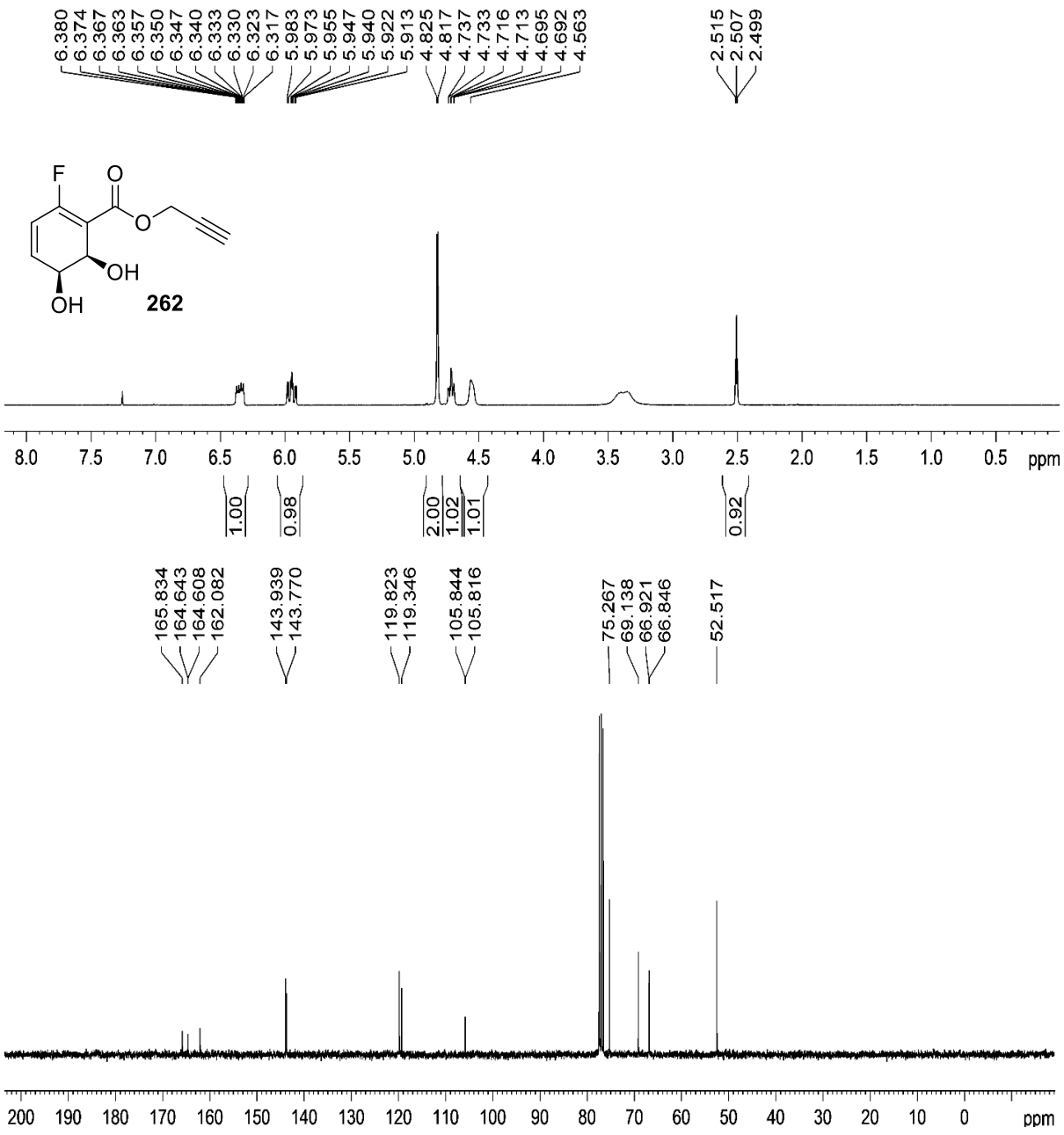
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6.330
6.323
6.317
5.983
5.973
5.955
5.947
5.940
5.922
5.913
4.825
4.817
4.737
4.733
4.716
4.713
4.695
4.692
4.563
2.515
2.507
2.499
F
O
O
OH
OH
262
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
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0.98
2.00
1.02
1.01
0.92
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164.608
162.082
143.939
143.770
119.823
119.346
105.844
105.816
75.267
69.138
66.921
66.846
52.517
200
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180
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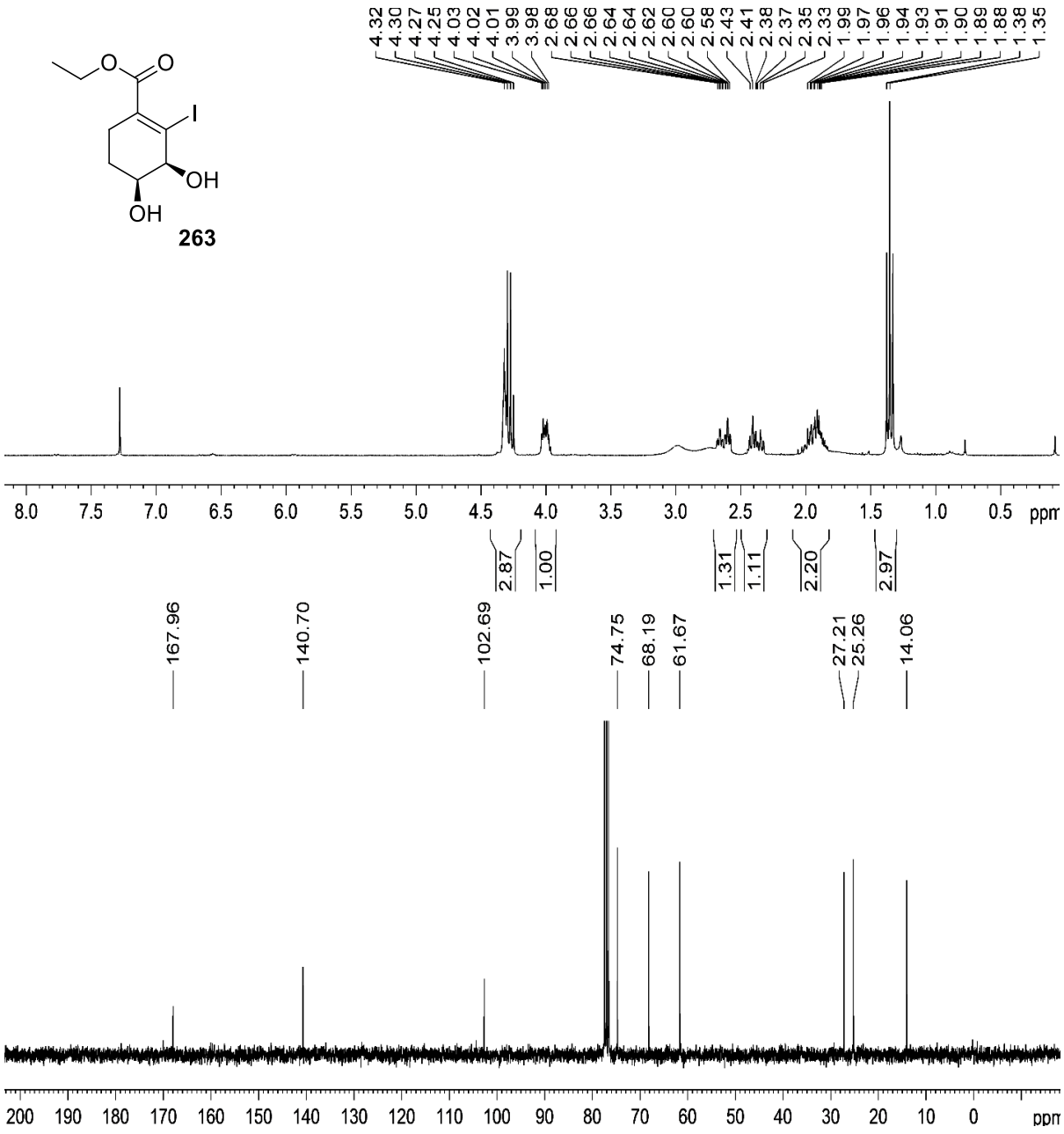
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm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4.02
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2.66
2.66
2.64
2.64
2.62
2.60
2.60
2.58
2.43
2.41
2.38
2.37
2.35
2.33
1.99
1.97
1.96
1.94
1.93
1.91
1.90
1.89
1.88
1.38
1.35
O
O
I
OH
OH
263
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7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
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1.31
1.11
2.20
2.97
167.96
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102.69
74.75
68.19
61.67
27.21
25.26
14.06
200
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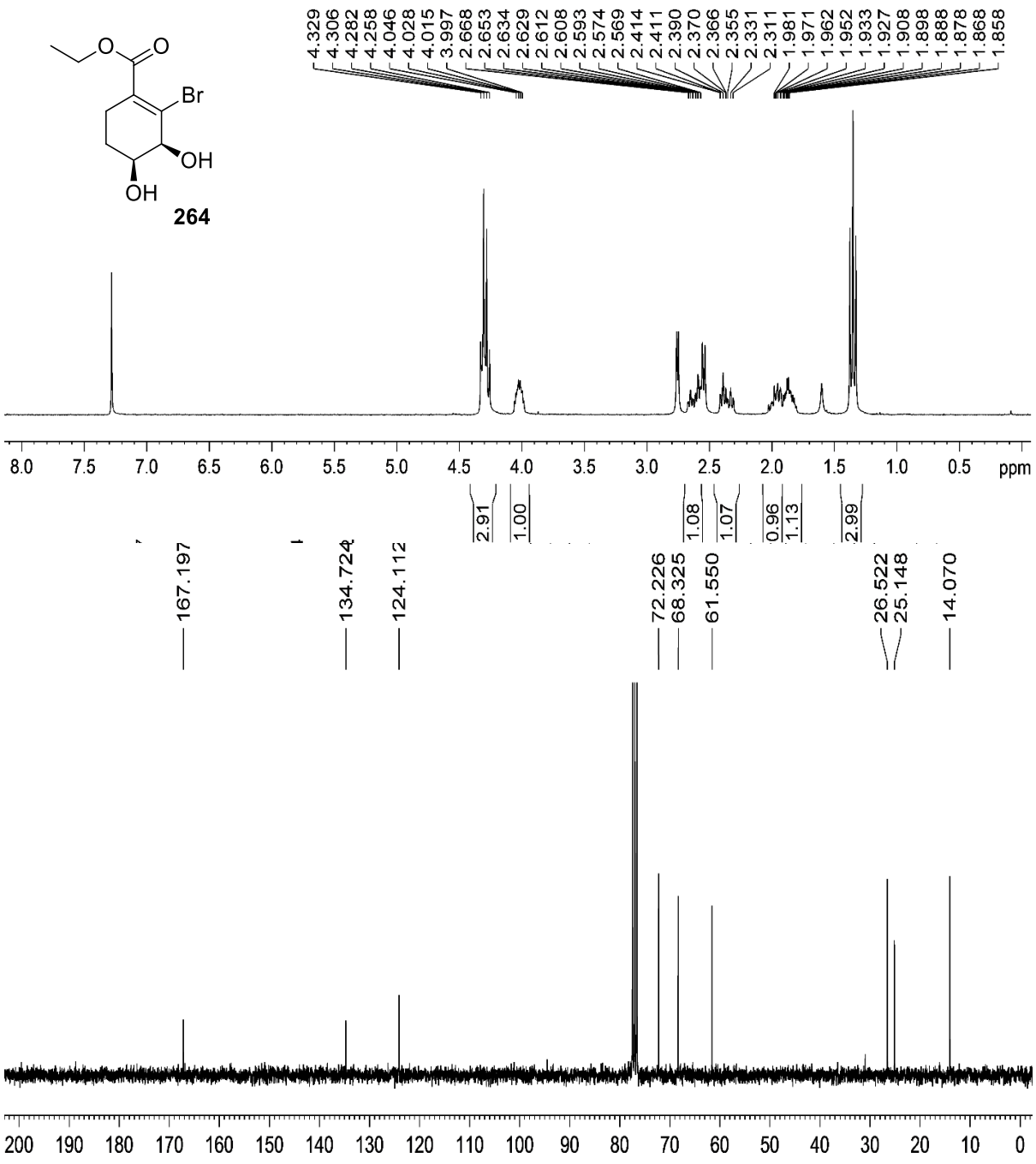
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm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O
Br
OH
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4.015
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2.653
2.634
2.629
2.612
2.608
2.593
2.574
2.569
2.414
2.411
2.390
2.370
2.366
2.355
2.331
2.311
1.981
1.971
1.962
1.952
1.933
1.927
1.908
1.898
1.888
1.878
1.868
1.858
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7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
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1.08
1.07
0.96
1.13
2.99
167.197
134.724
124.112
72.226
68.325
61.550
26.522
25.148
14.070
200
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150
140
130
120
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30
20
10
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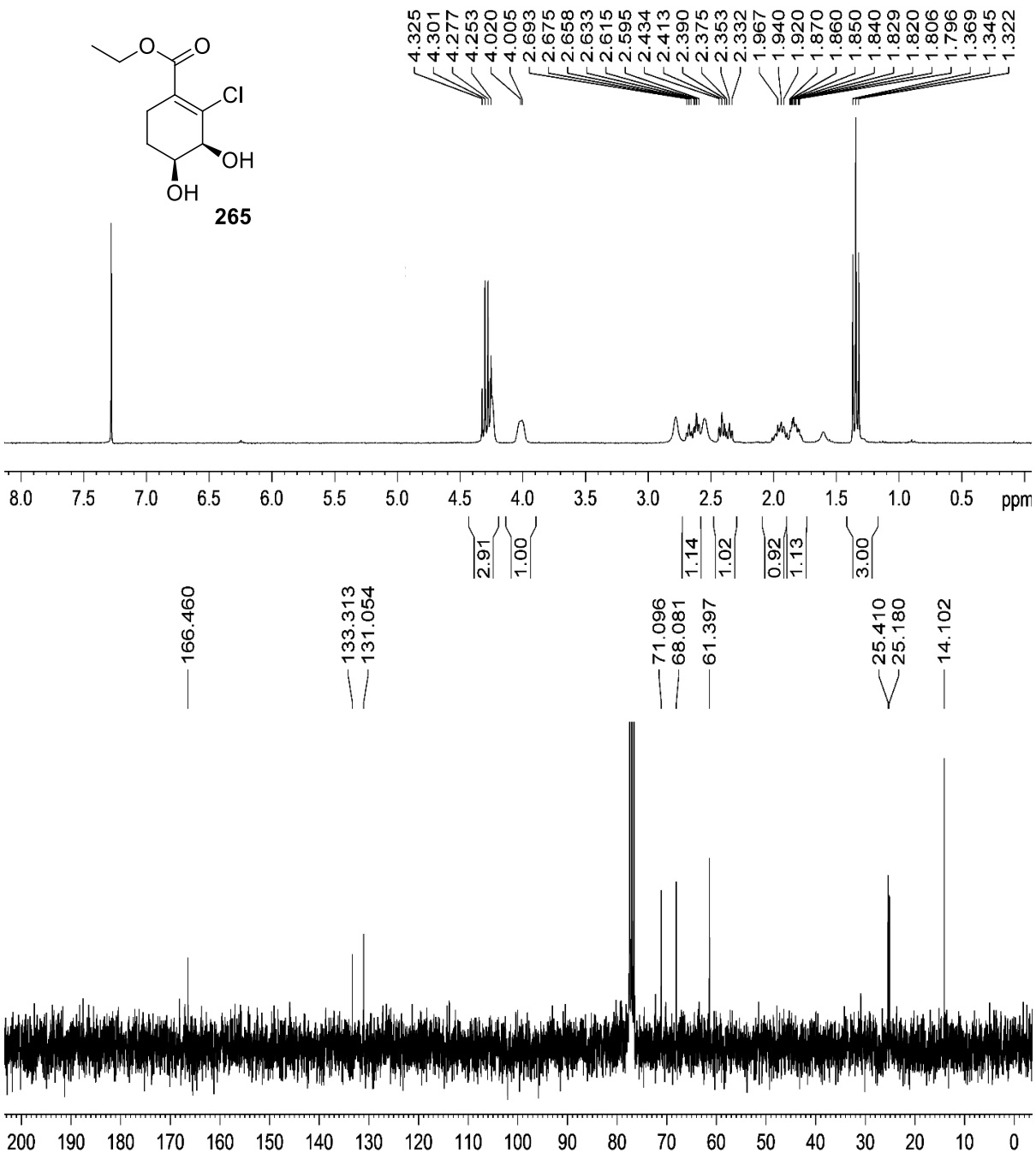
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2.434
2.413
2.390
2.375
2.353
2.332
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1.940
1.920
1.870
1.860
1.850
1.840
1.829
1.820
1.806
1.796
1.369
1.345
1.322
Cl
OH
OH
265
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2.5
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1.5
1.0
0.5
ppm
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1.02
0.92
1.13
3.00
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133.313
131.054
71.096
68.081
61.397
25.410
25.180
14.102
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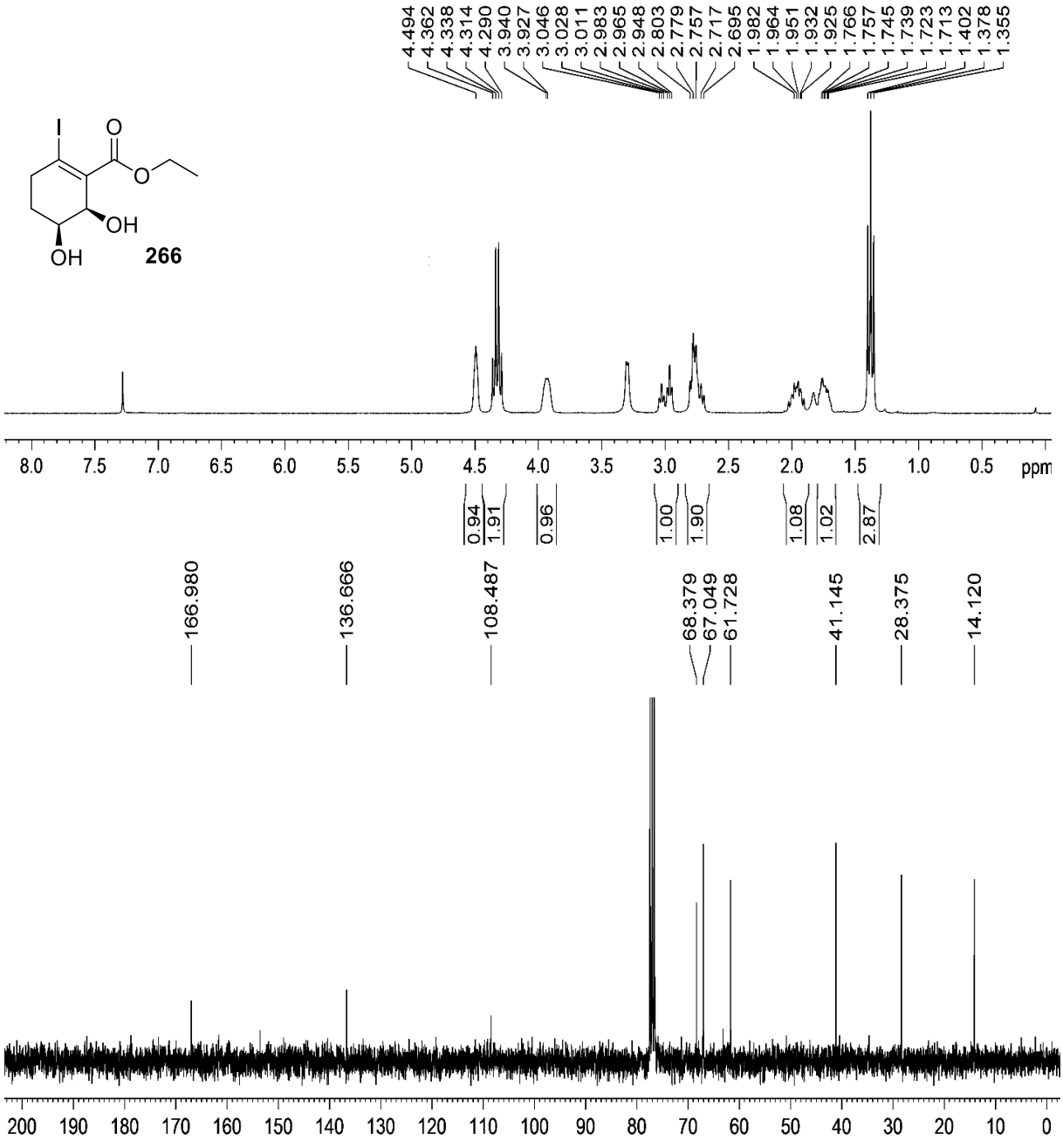
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3.011
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2.948
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2.779
2.757
2.717
2.695
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1.964
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1.932
1.925
1.766
1.757
1.745
1.739
1.723
1.713
1.402
1.378
1.355
I
O
O
OH
OH
266
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
0.94
1.91
0.96
1.00
1.90
1.08
1.02
2.87
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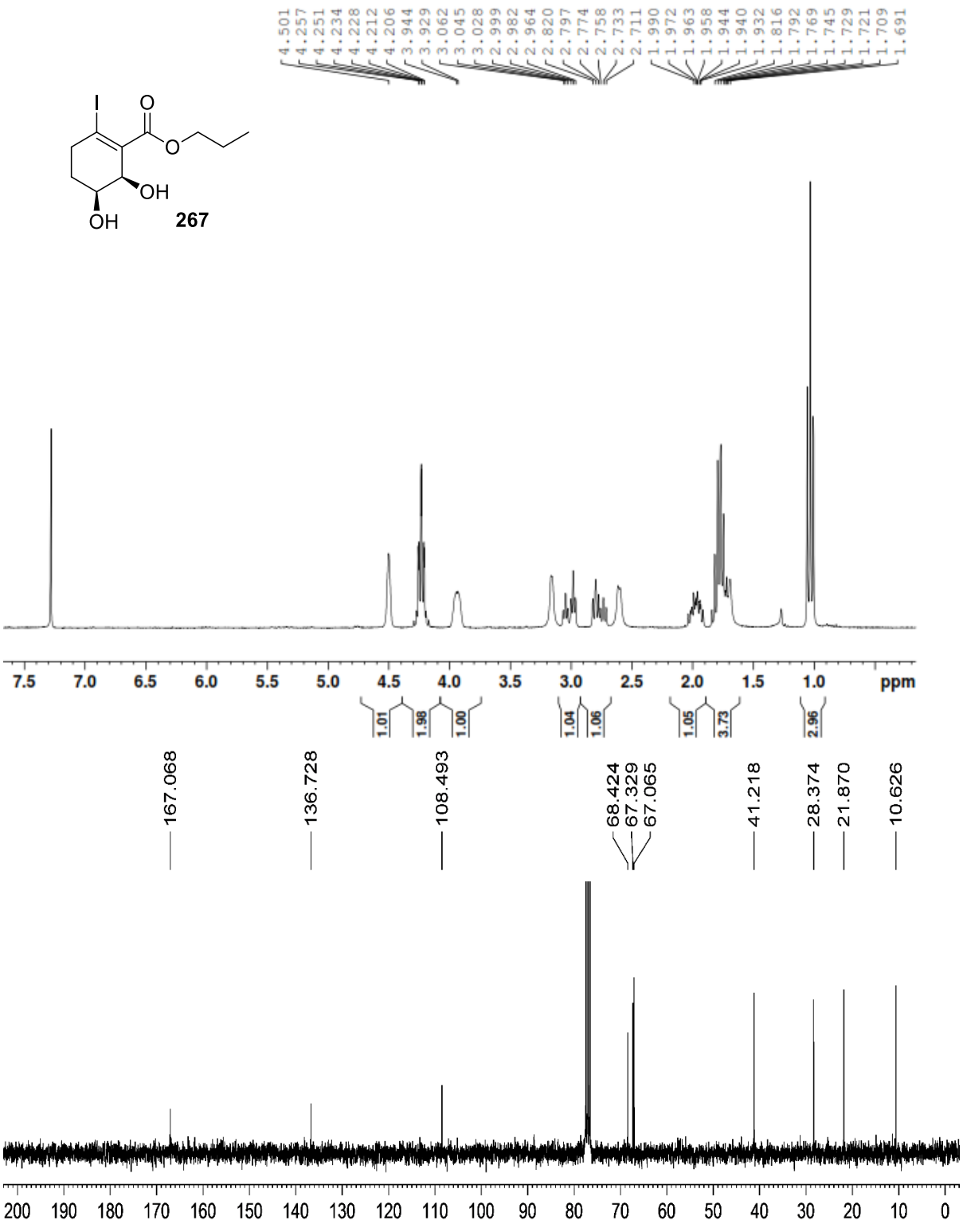
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3.028
2.999
2.982
2.964
2.820
2.797
2.774
2.758
2.733
2.711
1.990
1.972
1.963
1.958
1.944
1.940
1.932
1.816
1.792
1.769
1.745
1.729
1.721
1.709
1.691
I
O
O
OH
OH
267
7.5
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6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
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1.98
1.00
1.04
1.06
1.05
3.73
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67.329
67.065
41.218
28.374
21.870
10.626
200
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150
140
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120
110
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70
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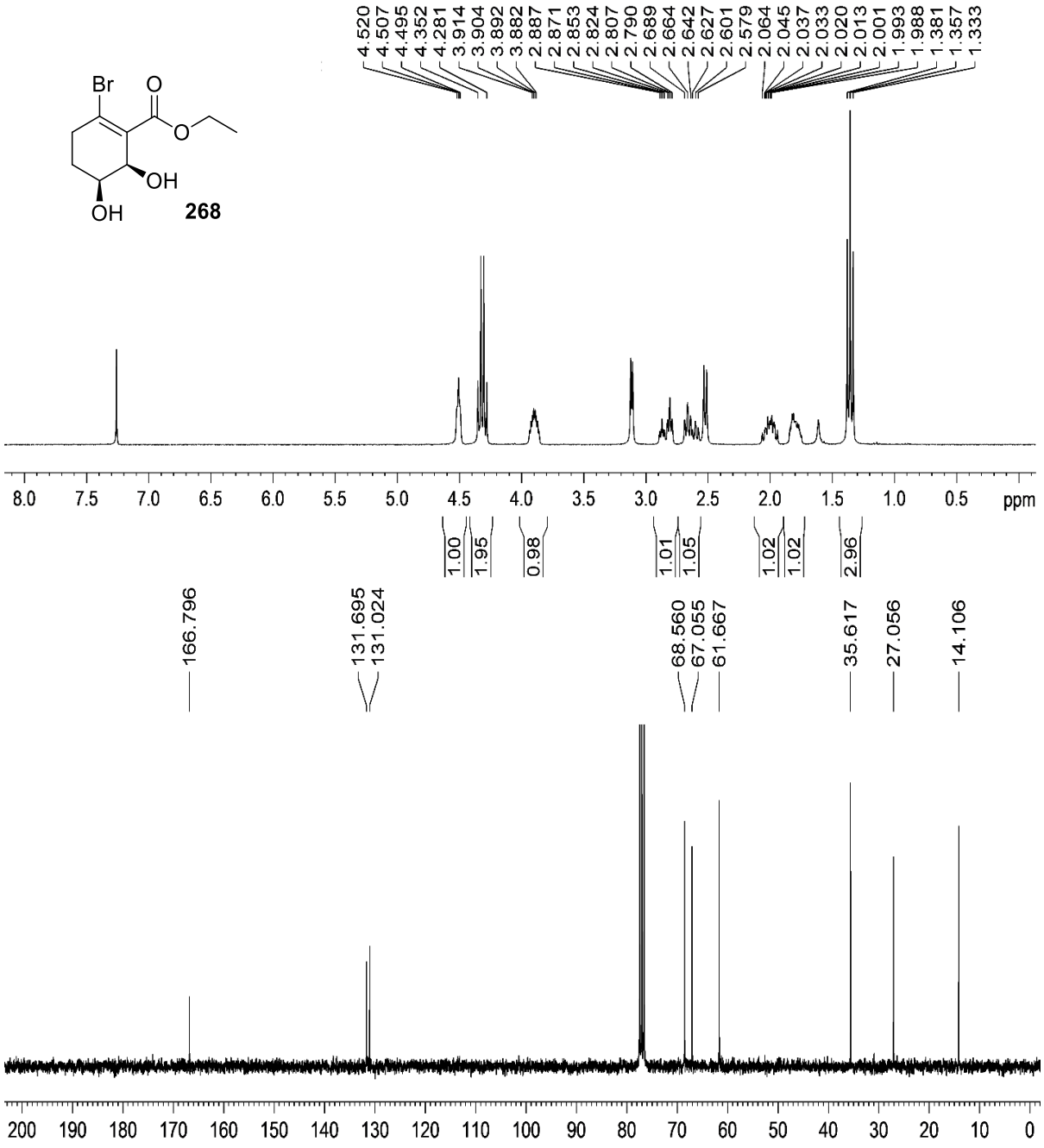
Br
O
O
OH
OH
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2.887
2.871
2.853
2.824
2.807
2.790
2.689
2.664
2.642
2.627
2.601
2.579
2.064
2.045
2.037
2.033
2.020
2.013
2.001
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1.988
1.381
1.357
1.333
8.0
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7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
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1.01
1.05
1.02
1.02
2.96
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14.106
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70
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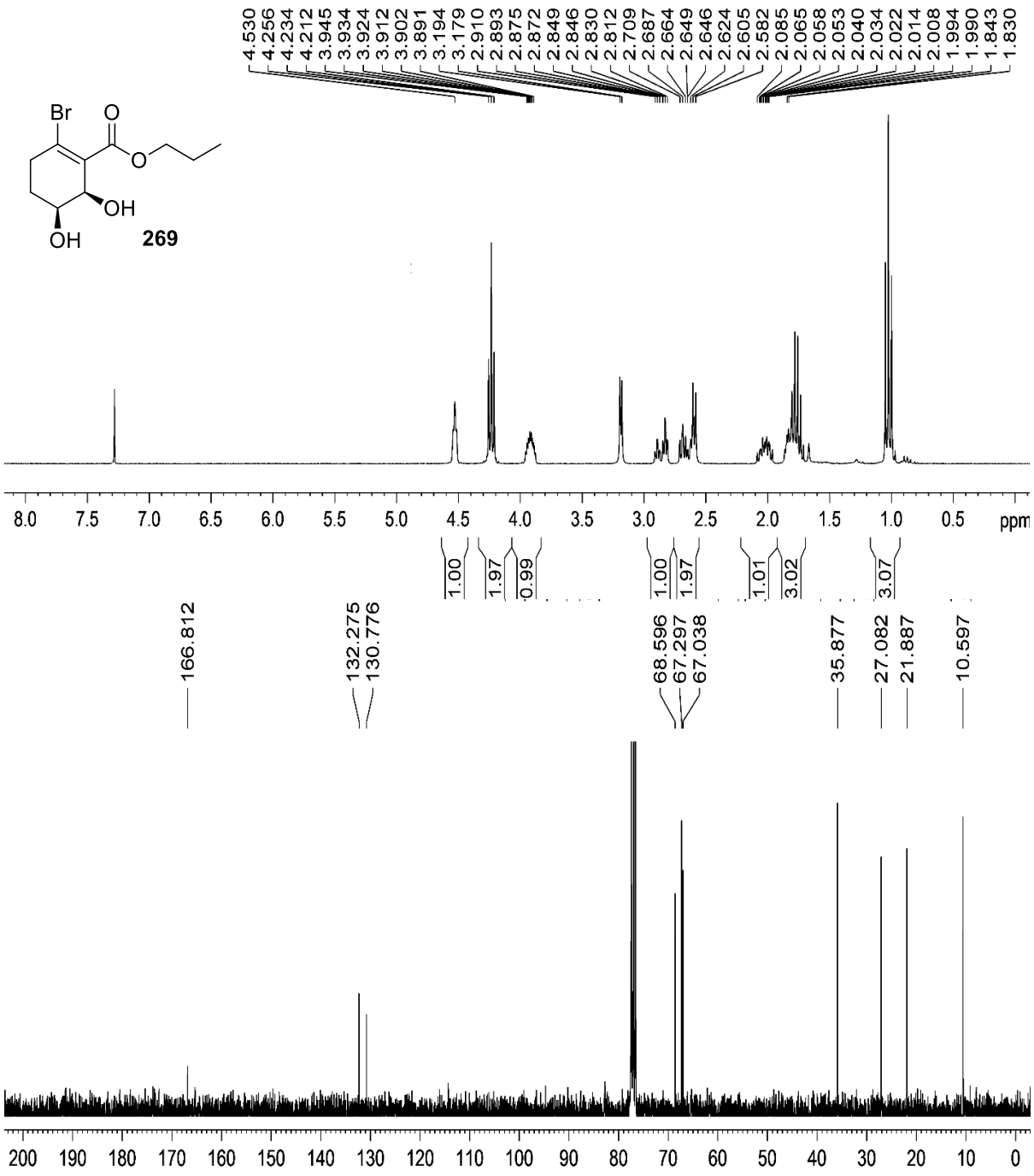
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2.910
2.893
2.875
2.872
2.849
2.846
2.830
2.812
2.709
2.687
2.664
2.649
2.646
2.624
2.605
2.582
2.085
2.065
2.058
2.053
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2.034
2.022
2.014
2.008
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1.990
1.843
1.830
Br
O
O
OH
OH
269
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7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
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2.0
1.5
1.0
0.5
ppm
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1.97
0.99
1.00
1.97
1.01
3.02
3.07
166.812
132.275
130.776
68.596
67.297
67.038
35.877
27.082
21.887
10.597
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70
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30
20
10
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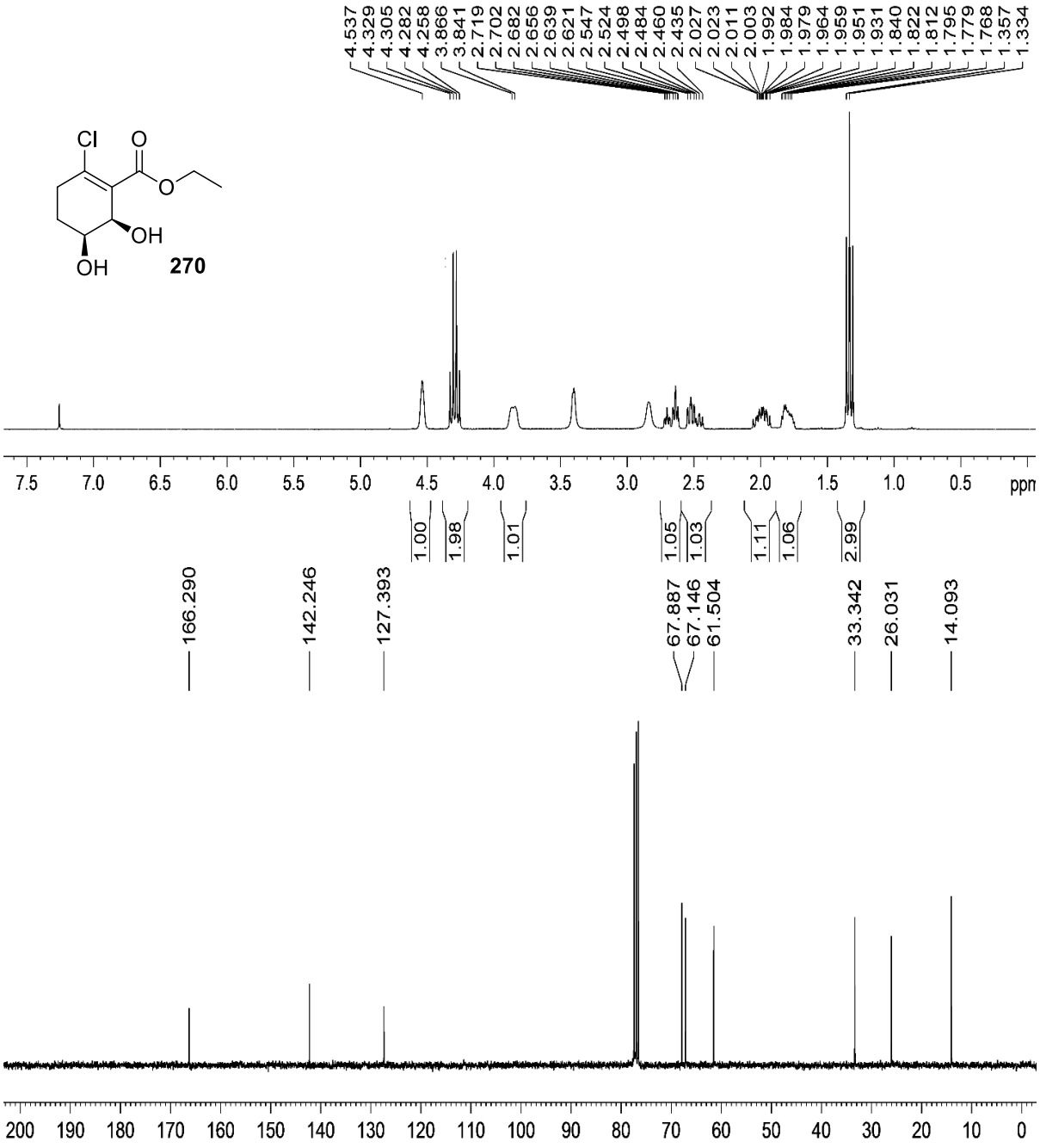
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2.621
2.547
2.524
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2.484
2.460
2.435
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2.023
2.011
2.003
1.992
1.984
1.979
1.964
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1.951
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1.812
1.795
1.779
1.768
1.357
1.334
Cl
O
O
OH
OH
270
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
1.00
1.98
1.01
1.05
1.03
1.11
1.06
2.99
166.290
142.246
127.393
67.887
67.146
61.504
33.342
26.031
14.093
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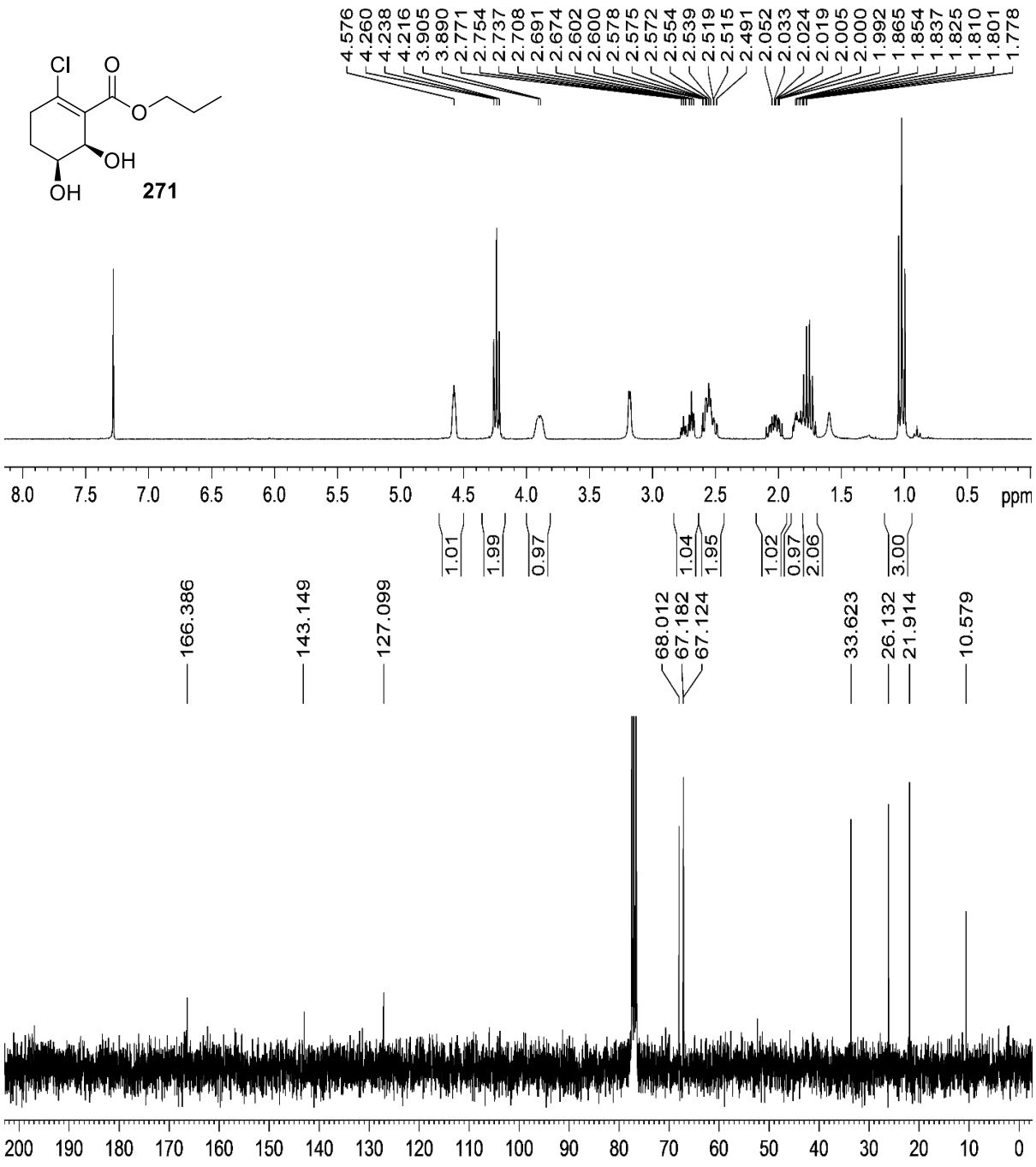
Cl
O
O
OH
OH
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4.260
4.238
4.216
3.905
3.890
2.771
2.754
2.737
2.708
2.691
2.674
2.602
2.600
2.578
2.575
2.572
2.554
2.539
2.519
2.515
2.491
2.052
2.033
2.024
2.019
2.005
2.000
1.992
1.865
1.854
1.837
1.825
1.810
1.801
1.778
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
1.01
1.99
0.97
1.04
1.95
1.02
0.97
2.06
3.00
166.386
143.149
127.099
68.012
67.182
67.124
33.623
26.132
21.914
10.579
200
190
180
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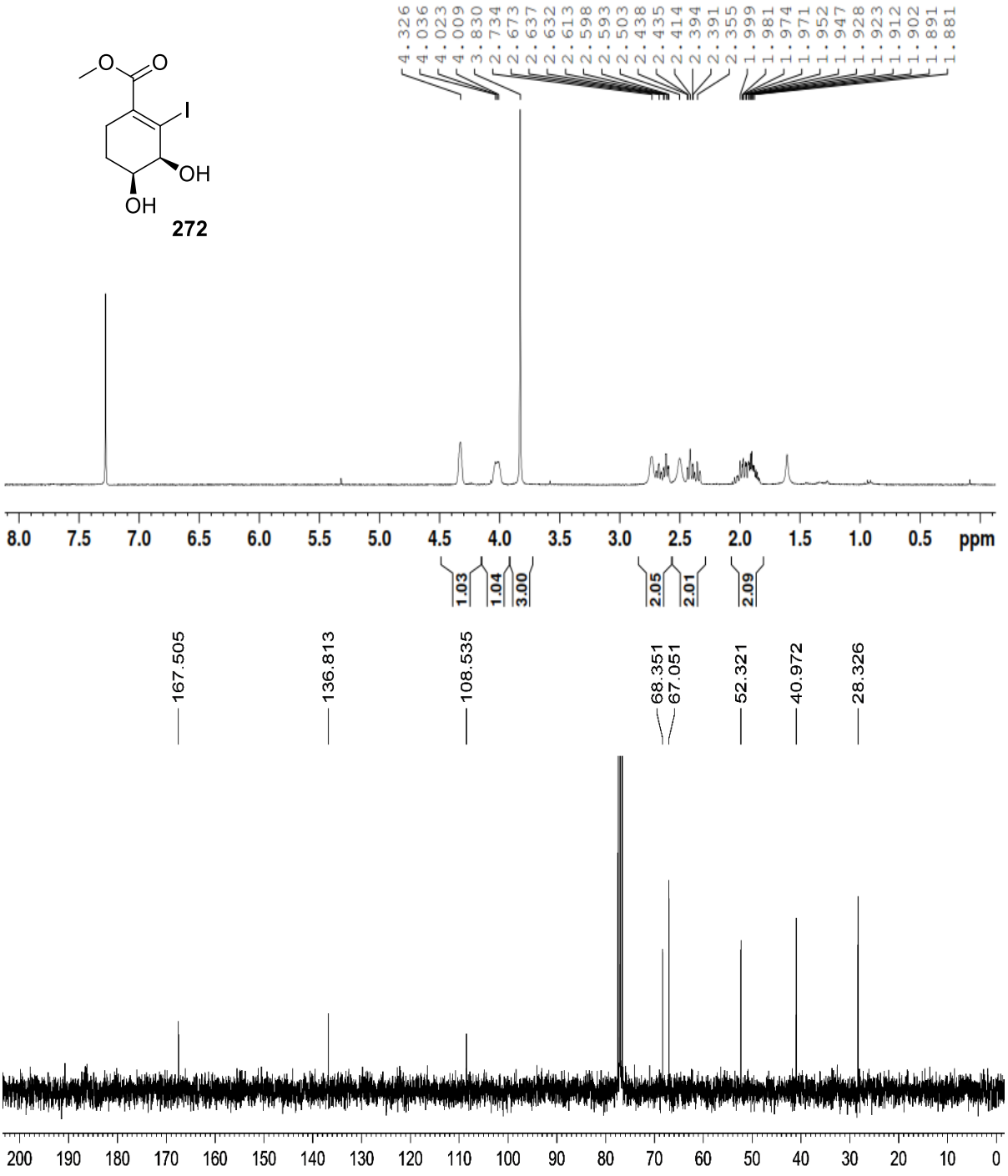
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0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4.036
4.023
4.009
3.830
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2.673
2.637
2.632
2.613
2.598
2.593
2.503
2.438
2.435
2.414
2.394
2.391
2.355
1.999
1.981
1.974
1.971
1.952
1.947
1.928
1.923
1.912
1.902
1.891
1.881
O
O
I
OH
OH
272
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
1.03
1.04
3.00
2.05
2.01
2.09
167.505
136.813
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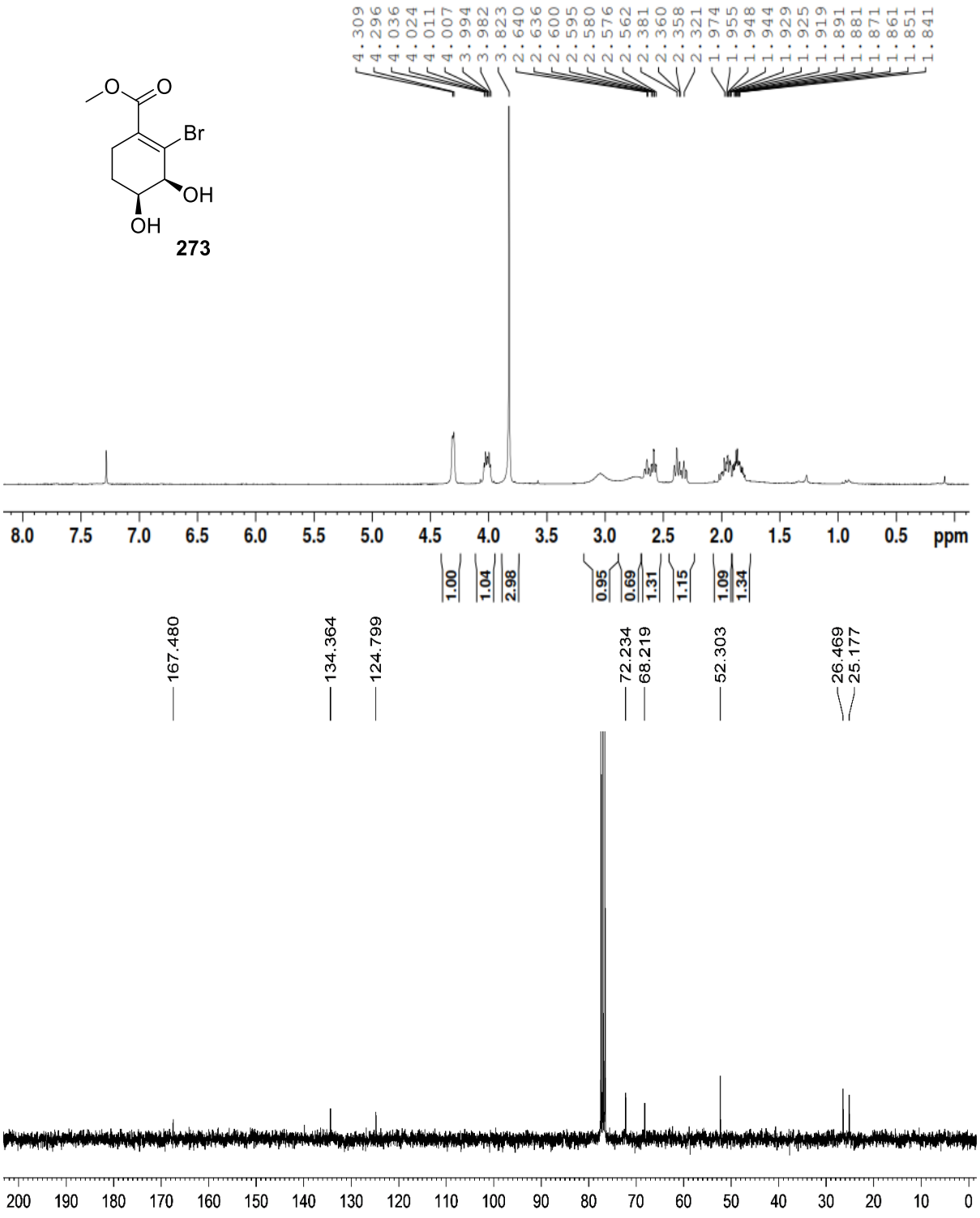
68.351
67.051
52.321
40.972
28.326
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0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4.036
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4.007
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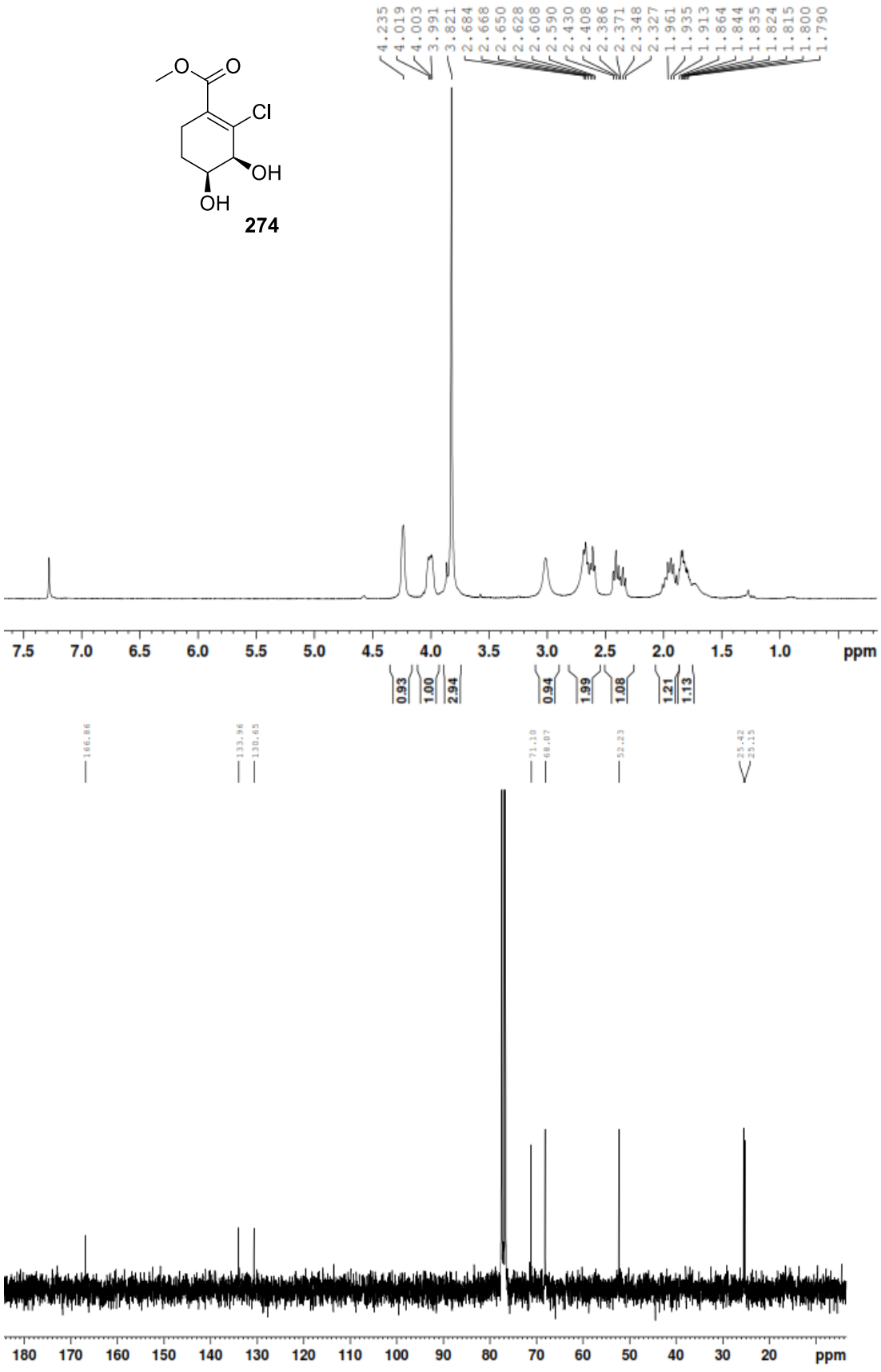
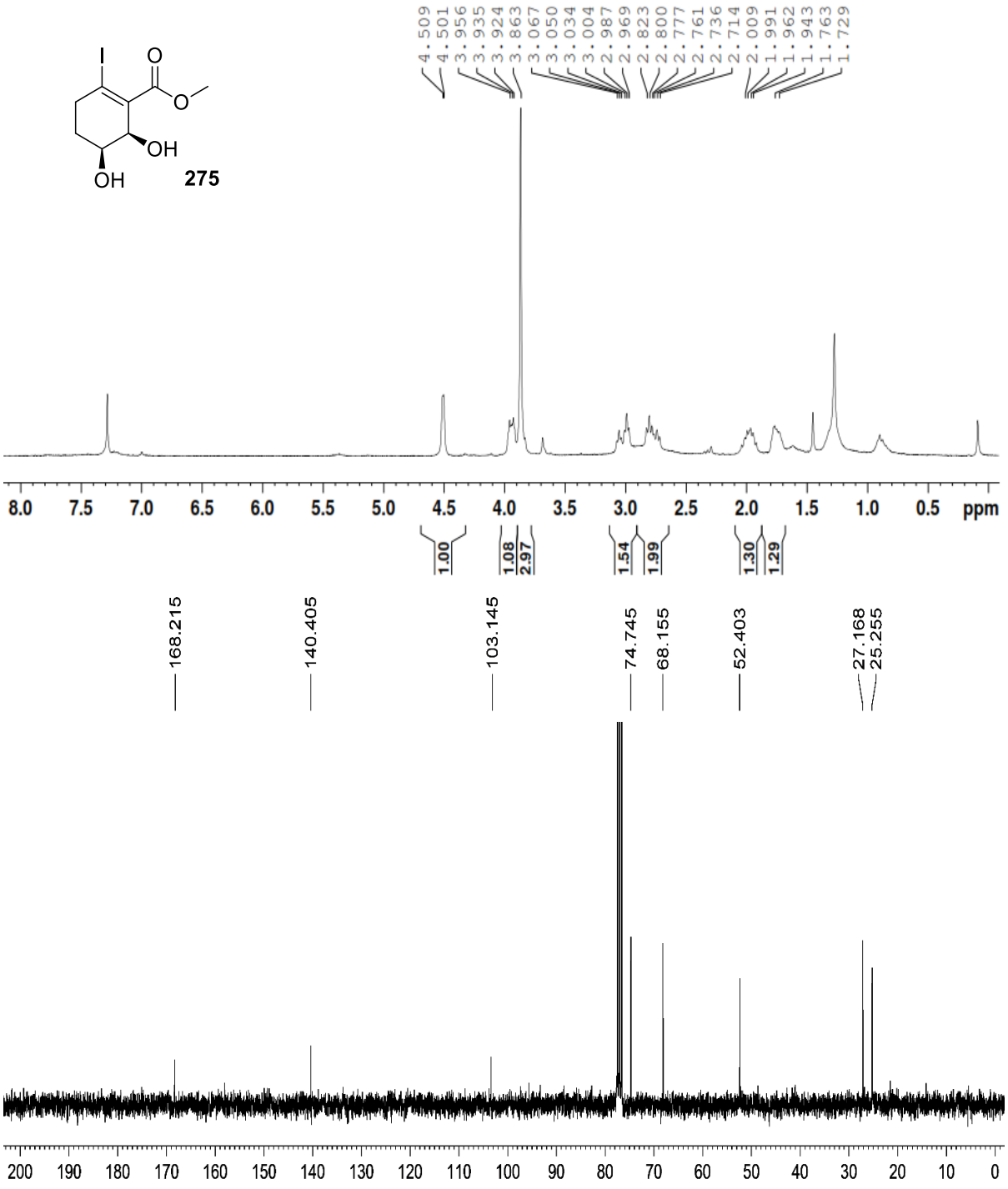
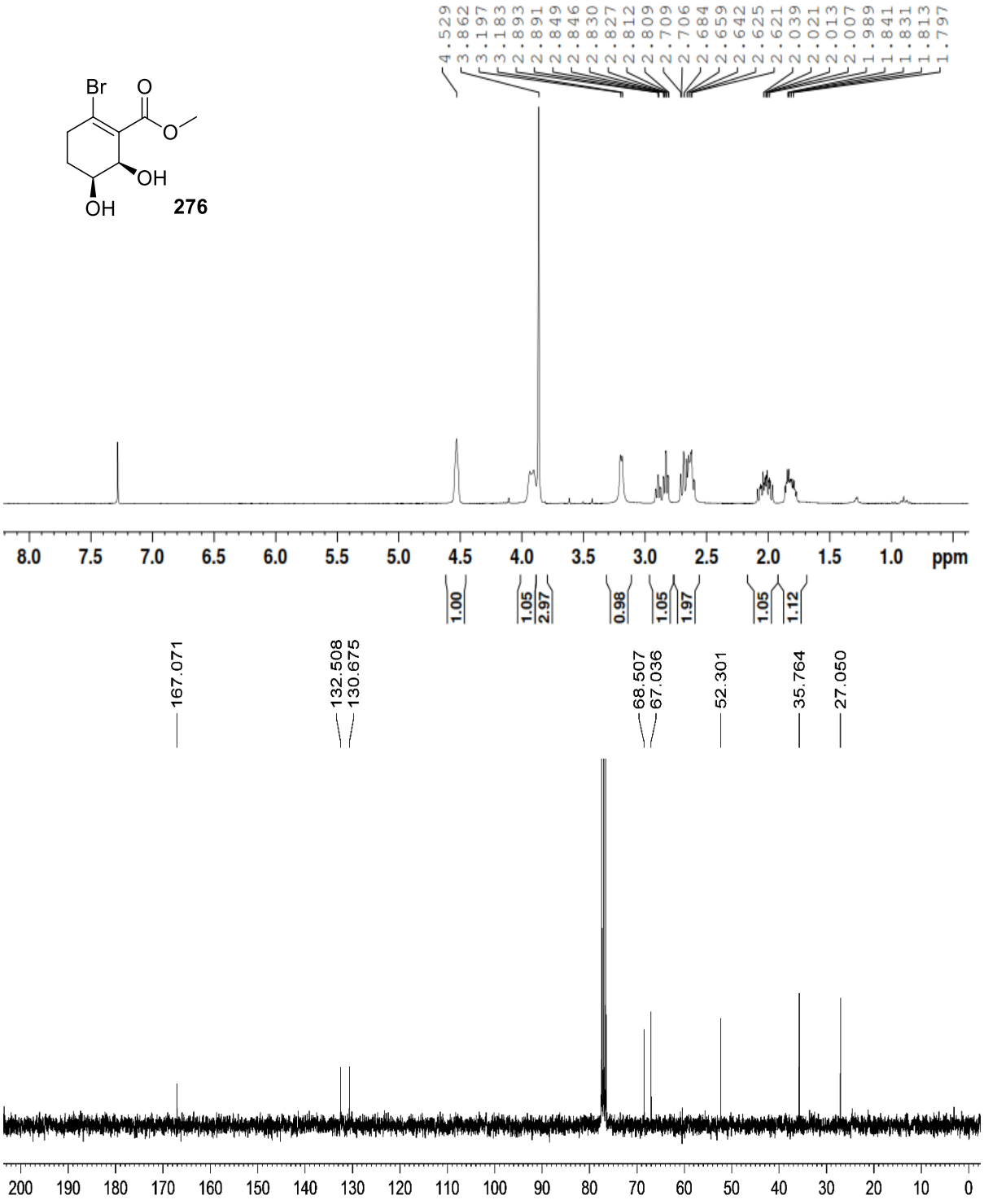
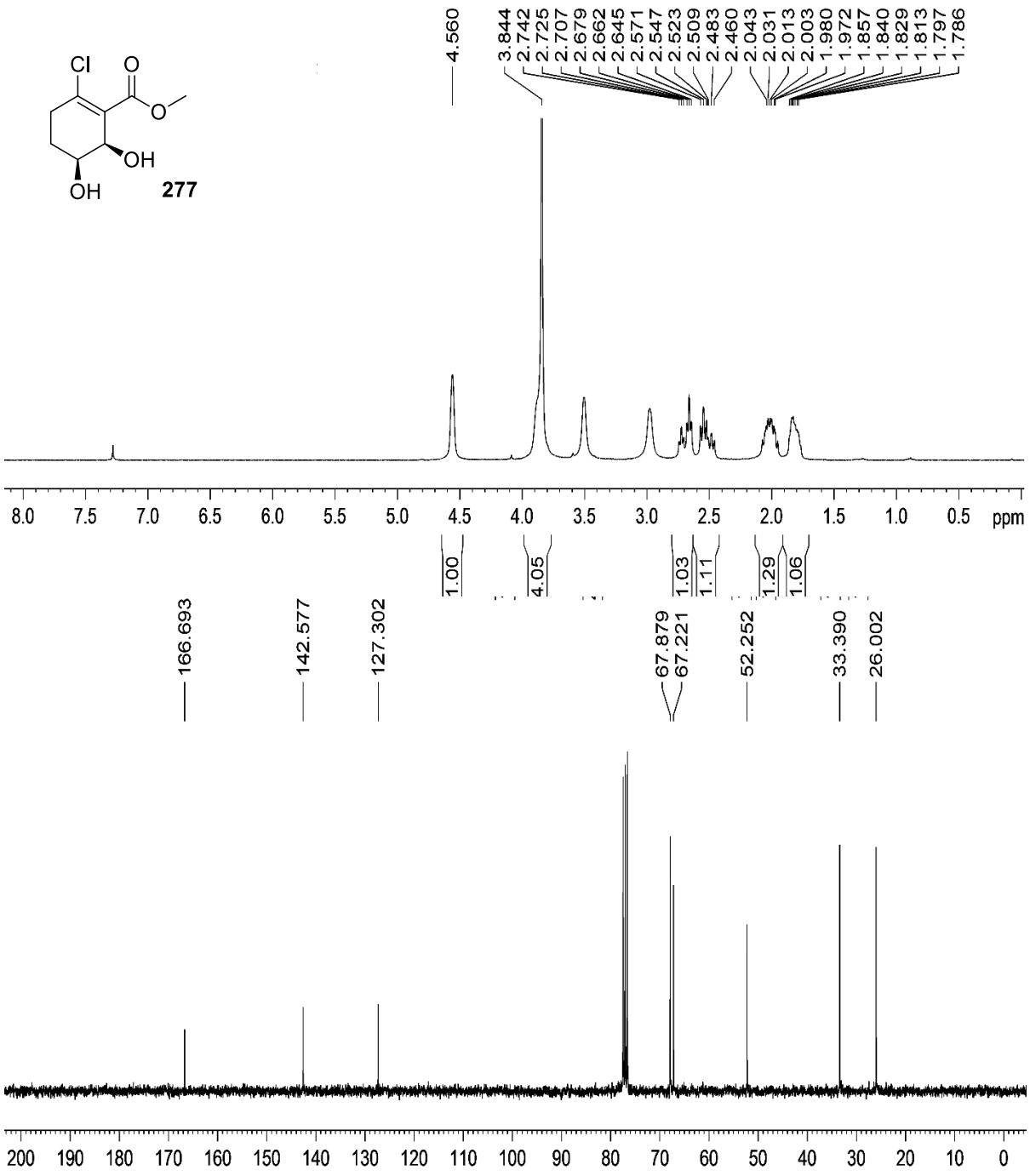
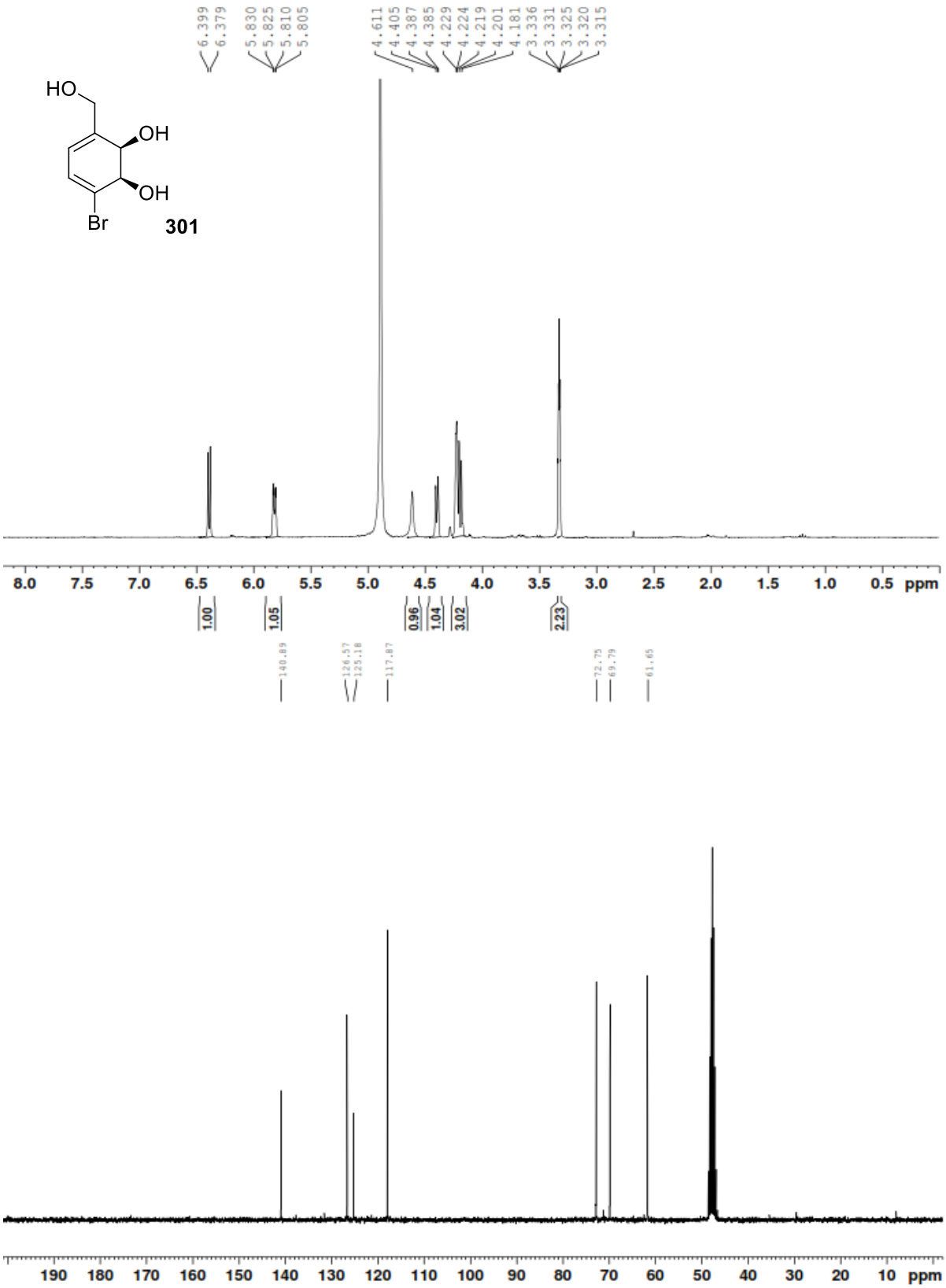
3.982
3.823
2.640
2.636
2.600
2.595
2.580
2.576
2.562
2.381
2.360
2.358
2.321
1.974
1.955
1.948
1.944
1.929
1.925
1.919
1.891
1.881
1.871
1.861
1.851
1.841
O
O
Br
OH
OH
273
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
1.00
1.04
2.98
0.95
0.69
1.31
1.15
1.09
1.34
167.480
134.364
124.799
72.234
68.219
52.303
26.469
25.177
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

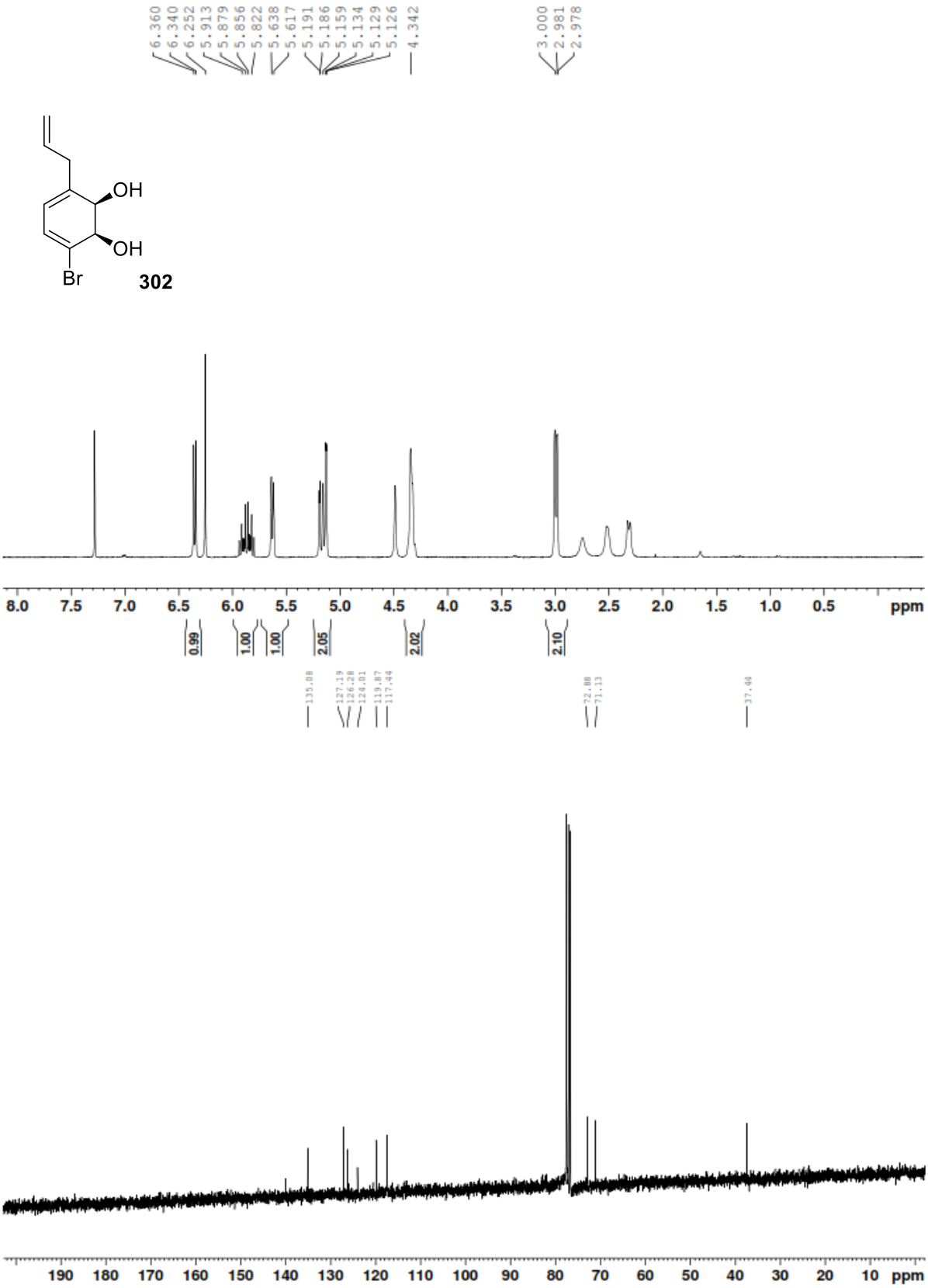

4.235
4.019
4.003
3.991
3.821
2.684
2.668
2.650
2.628
2.608
2.590
2.430
2.408
2.386
2.371
2.348
2.327
1.961
1.935
1.913
1.864
1.844
1.835
1.824
1.815
1.800
1.790
O
O
Cl
OH
OH
274
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm
0.93 1.00 2.94 0.94 1.99 1.08 1.21 1.13
166.86
133.96
130.65
71.10
68.07
52.23
25.42
25.15
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

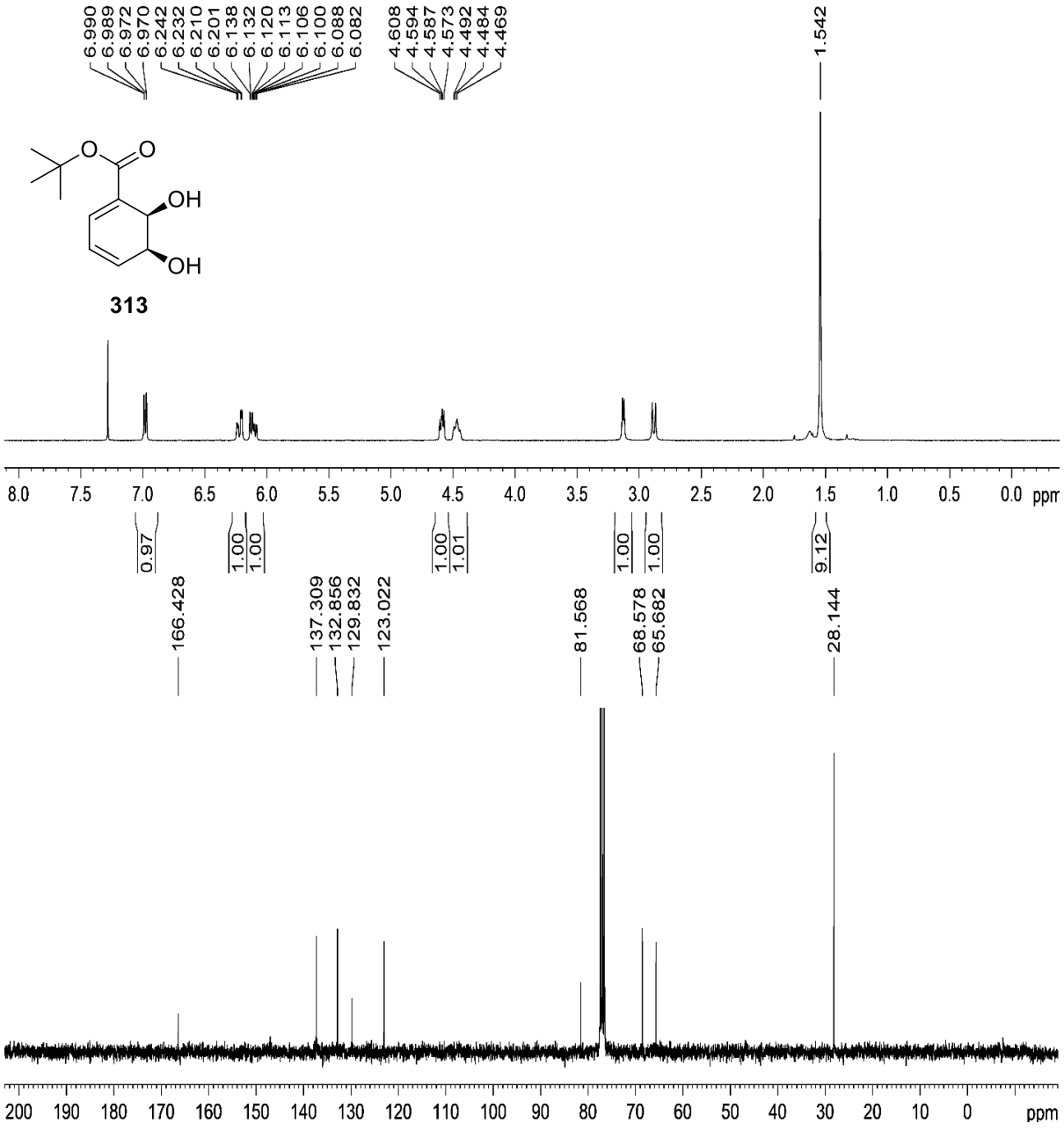

I
O
O
OH
OH
275
4.509
4.501
3.956
3.935
3.924
3.863
3.067
3.050
3.034
3.004
2.987
2.969
2.823
2.800
2.777
2.761
2.736
2.714
2.009
1.991
1.962
1.943
1.763
1.729
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
1.00
1.08
2.97
1.54
1.99
1.30
1.29
168.215
140.405
103.145
74.745
68.155
52.403
27.168
25.255
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0


Br
O
O
OH
OH
276
4.529
3.862
3.197
3.183
2.893
2.891
2.849
2.846
2.830
2.827
2.812
2.809
2.709
2.706
2.684
2.659
2.642
2.625
2.621
2.039
2.021
2.013
2.007
1.989
1.841
1.831
1.813
1.797
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm
1.00
1.05
2.97
0.98
1.05
1.97
1.05
1.12
167.071
132.508
130.675
68.507
67.036
52.301
35.764
27.050
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0


Cl
O
O
OH
OH
277
4.560
3.844
2.742
2.725
2.707
2.679
2.662
2.645
2.571
2.547
2.523
2.509
2.483
2.460
2.043
2.031
2.013
2.003
1.980
1.972
1.857
1.840
1.829
1.813
1.797
1.786
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
1.00
4.05
1.03
1.11
1.29
1.06
166.693
142.577
127.302
67.879
67.221
52.252
33.390
26.002
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0


HO
OH
OH
Br
301
6.399
6.379
5.830
5.825
5.810
5.805
4.611
4.405
4.387
4.385
4.229
4.224
4.219
4.201
4.181
3.336
3.331
3.325
3.320
3.315
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
1.00
1.05
0.96
1.04
3.02
2.23
140.89
126.57
125.18
117.87
72.75
69.79
61.65
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm


6.360
6.340
6.252
5.913
5.879
5.856
5.822
5.638
5.617
5.191
5.186
5.159
5.134
5.129
5.126
4.342
3.000
2.981
2.978
OH
OH
Br
302
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
0.99
1.00
1.00
2.05
2.02
2.10
135.08
127.19
126.28
124.01
119.87
117.44
72.88
71.13
37.44
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm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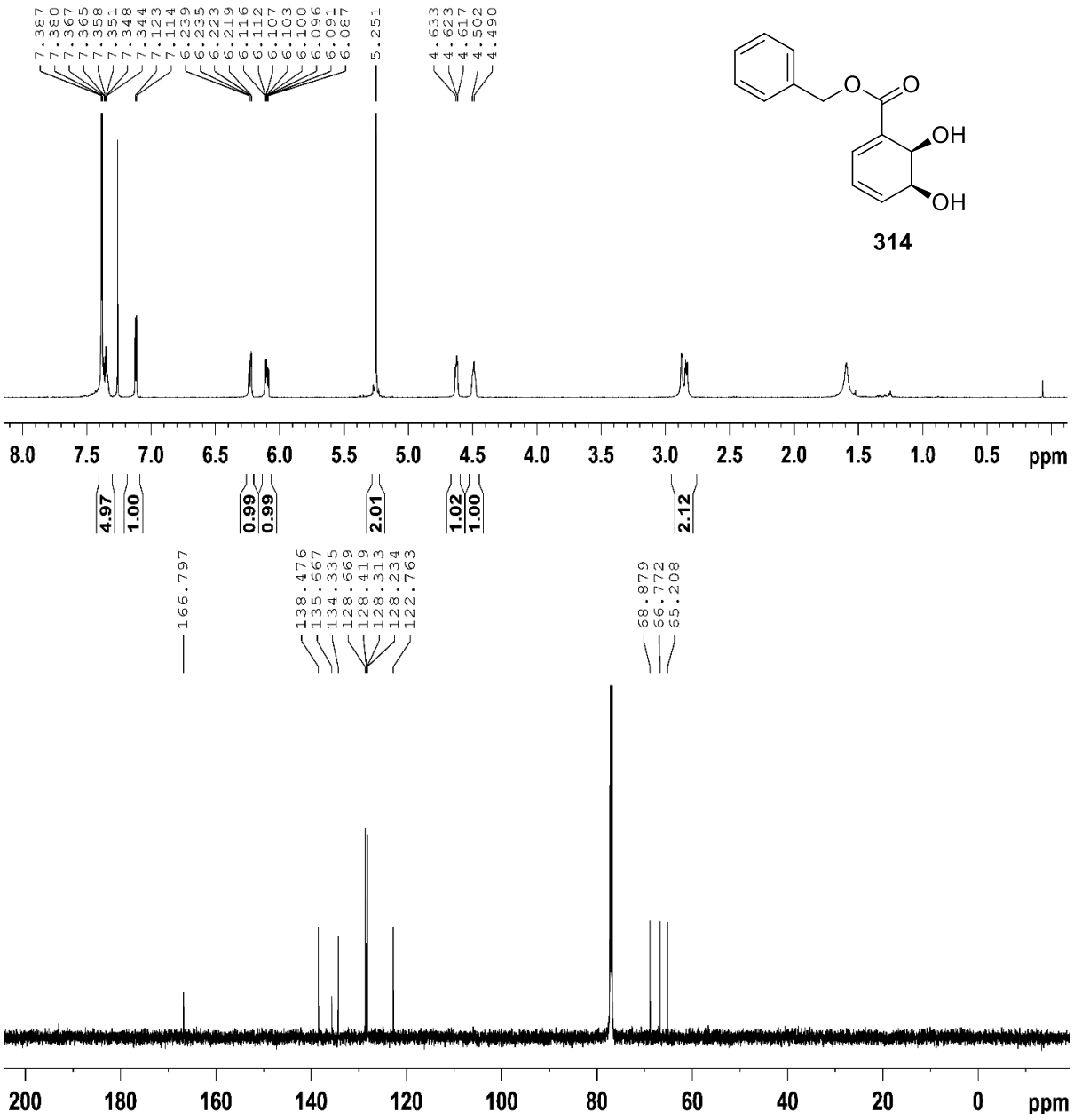
6.989
6.972
6.970
6.242
6.232
6.210
6.201
6.138
6.132
6.120
6.113
6.106
6.100
6.088
6.082
4.608
4.594
4.587
4.573
4.492
4.484
4.469
1.542
O
O
OH
OH
313
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm
0.97
1.00
1.00
1.00
1.01
1.00
1.00
9.12
166.428
137.309
132.856
129.832
123.022
81.568
68.578
65.682
28.144
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm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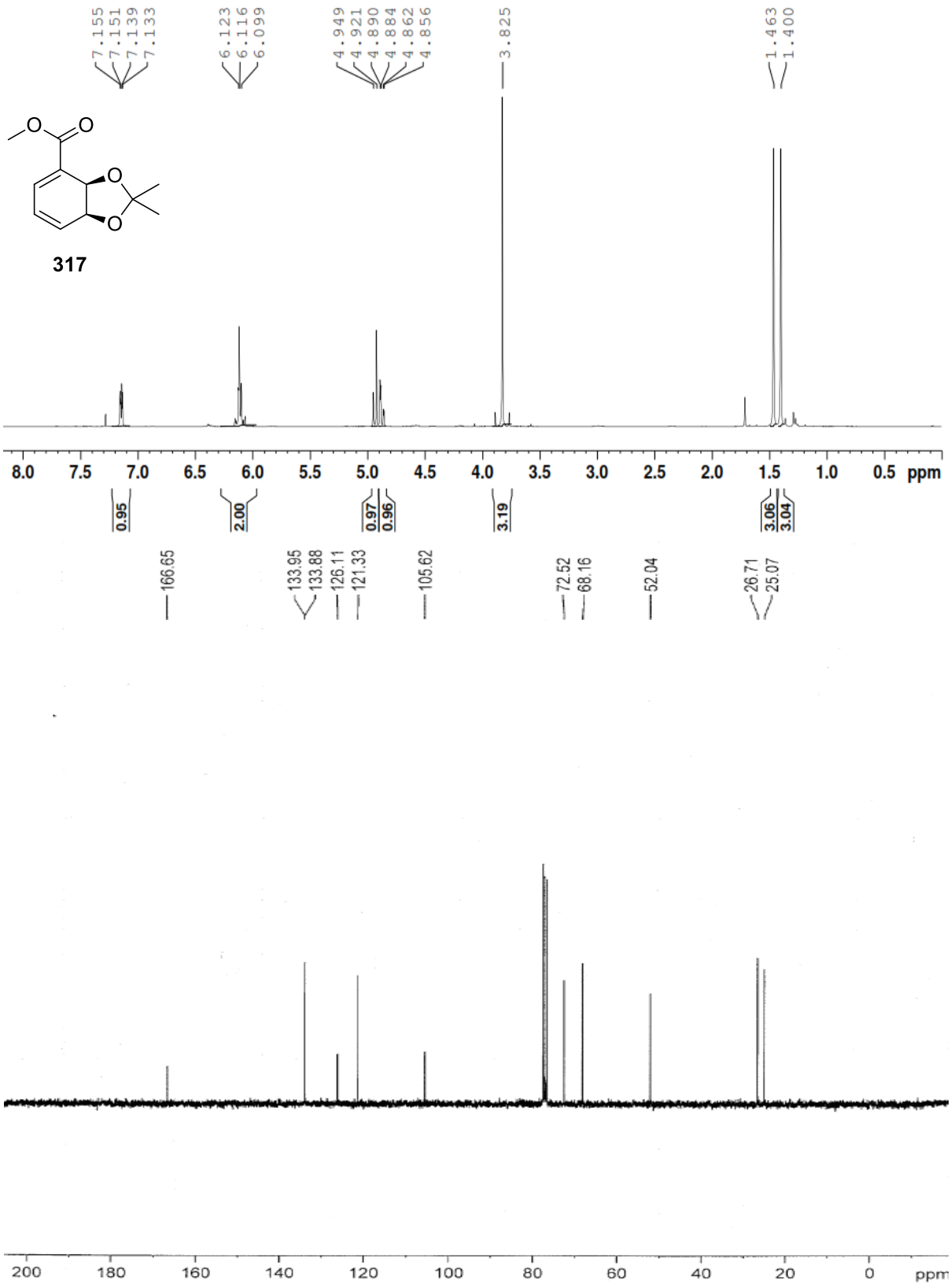
7.367
7.365
7.358
7.351
7.348
7.344
7.123
7.114
6.239
6.235
6.223
6.219
6.116
6.112
6.107
6.103
6.100
6.096
6.091
6.087
5.251
4.633
4.623
4.617
4.502
4.490
O
O
OH
OH
314
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
4.97
1.00
0.99
0.99
2.01
1.02
1.00
2.12
166.797
138.476
135.667
134.335
128.669
128.419
128.313
128.234
122.763
68.879
66.772
65.208
200
180
160
140
120
100
80
60
40
20
0
ppm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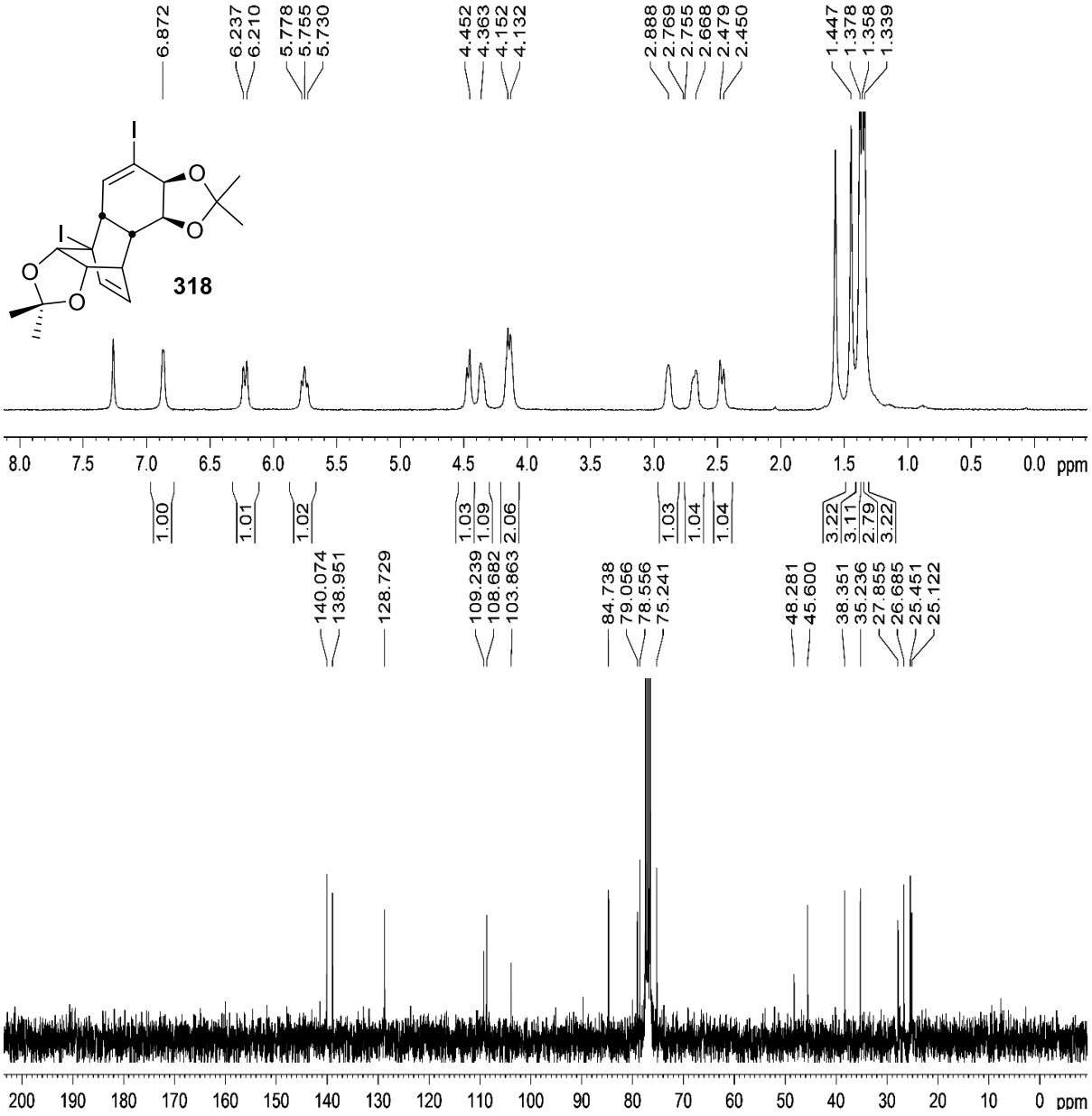
7.151
7.139
7.133
6.123
6.116
6.099
4.949
4.921
4.890
4.884
4.862
4.856
3.825
1.463
1.400
317
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
0.95
2.00
0.97
0.96
3.19
3.06
3.04
166.65
133.95
133.88
126.11
121.33
105.62
72.52
68.16
52.04
26.71
25.07
200 180 160 140 120 100 80 60 40 20 0 ppm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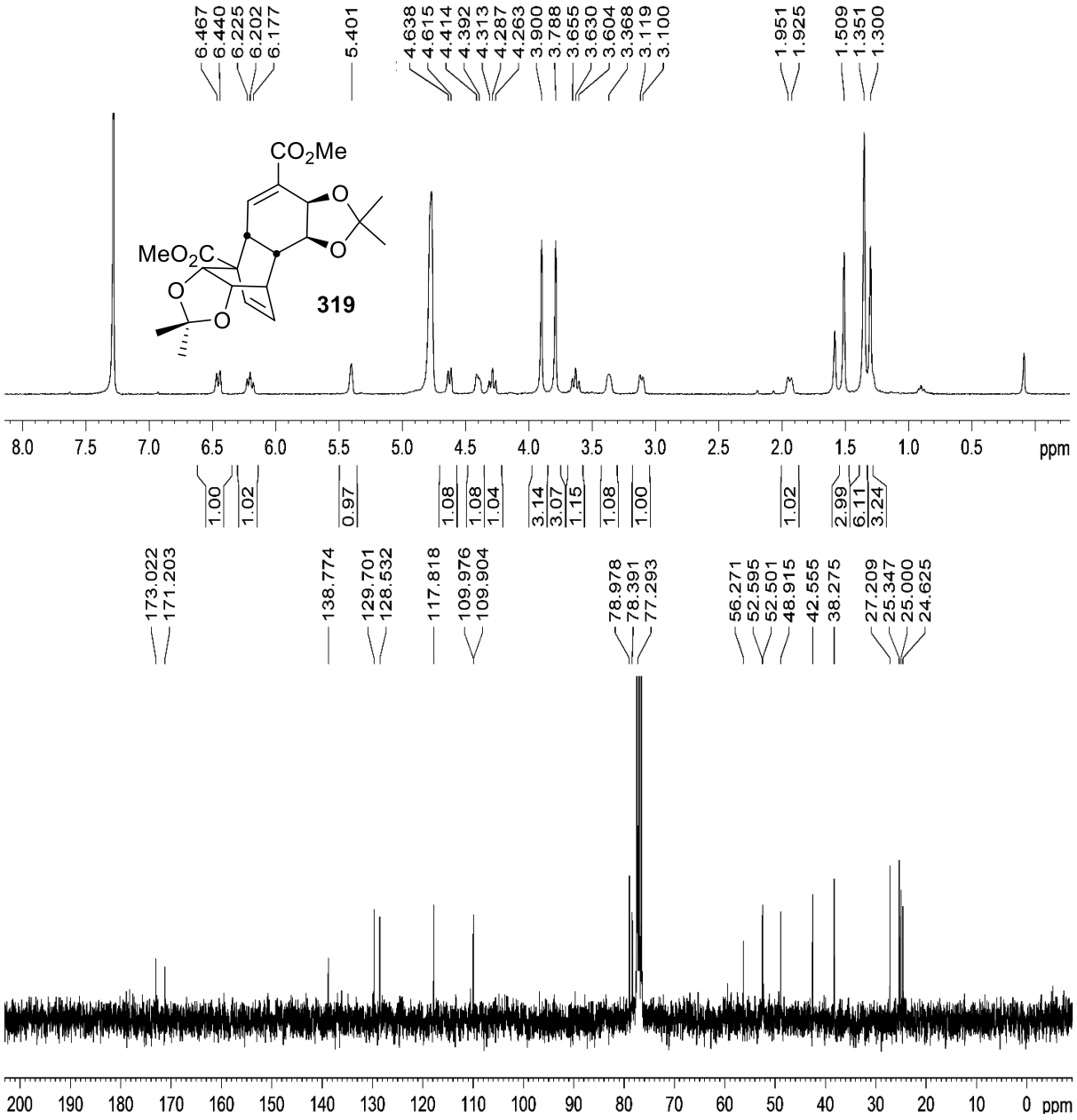
6.210
5.778
5.755
5.730
4.452
4.363
4.152
4.132
2.888
2.769
2.755
2.668
2.479
2.450
1.447
1.378
1.358
1.339
I
I
O
O
O
O
318
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm
1.00
1.01
1.02
1.03
1.09
2.06
1.03
1.04
1.04
3.22
3.11
2.79
3.22
140.074
138.951
128.729
109.239
108.682
103.863
84.738
79.056
78.556
75.241
48.281
45.600
38.351
35.236
27.855
26.685
25.451
25.122
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm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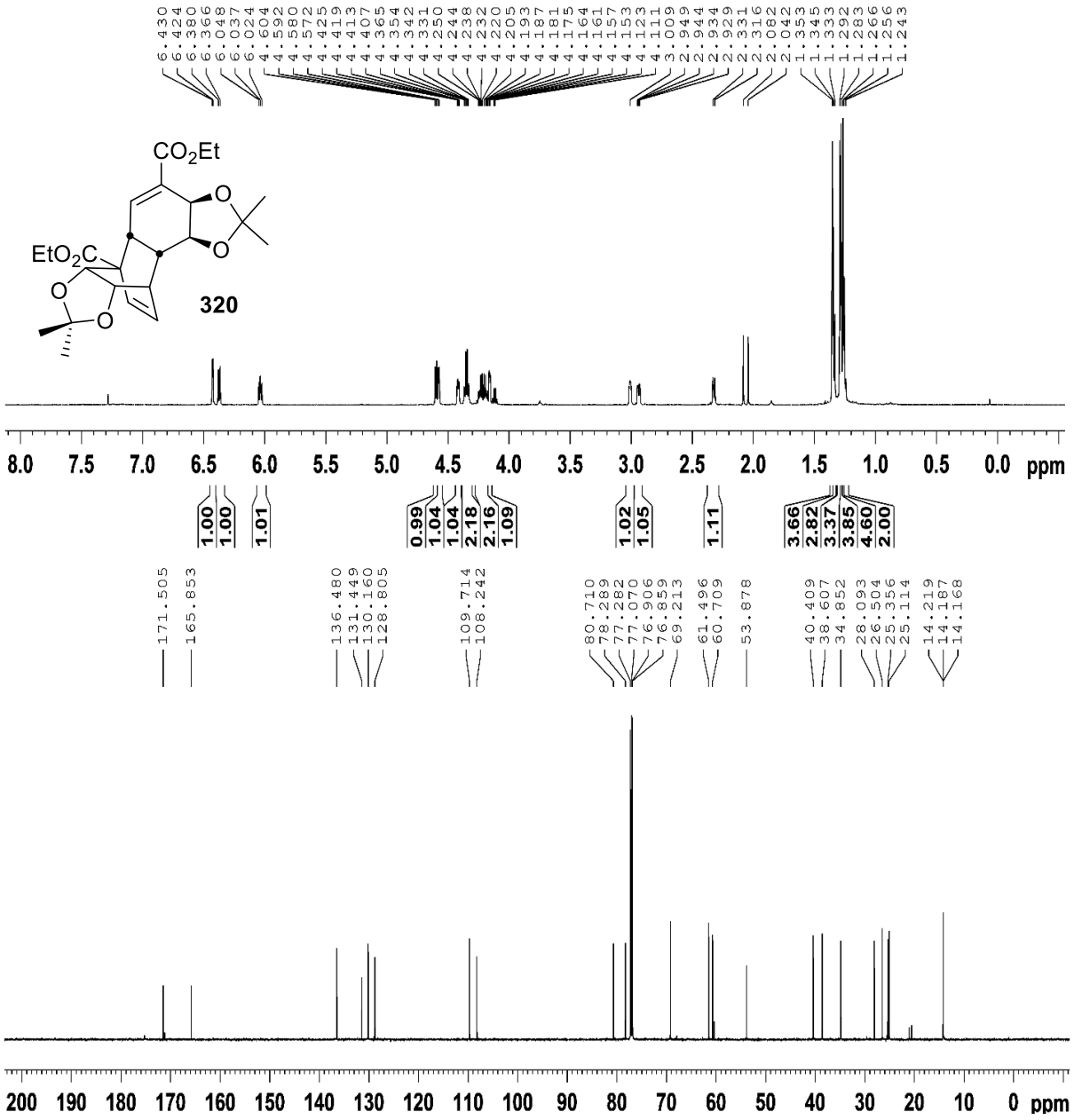
6.225
6.202
6.177
5.401
4.638
4.615
4.414
4.392
4.313
4.287
4.263
3.900
3.788
3.655
3.630
3.604
3.368
3.119
3.100
1.951
1.925
1.509
1.351
1.300
CO2Me
MeO2C
O
O
O
O
319
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
1.00
1.02
0.97
1.08
1.08
1.04
3.14
3.07
1.15
1.08
1.00
1.02
2.99
6.11
3.24
173.022
171.203
138.774
129.701
128.532
117.818
109.976
109.904
78.978
78.391
77.293
56.271
52.595
52.501
48.915
42.555
38.275
27.209
25.347
25.000
24.625
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm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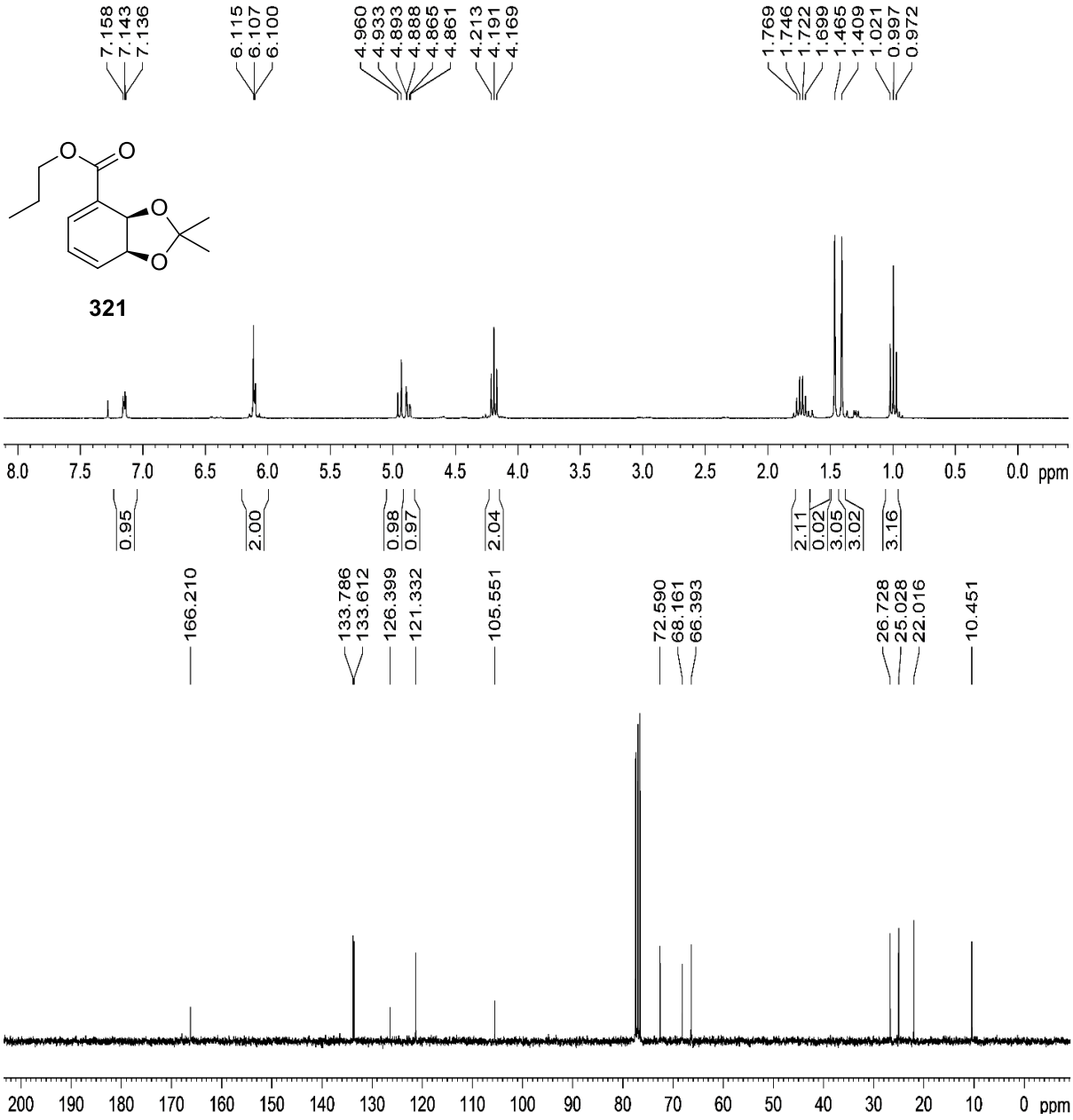
6.380
6.366
6.048
6.037
6.024
4.604
4.592
4.580
4.572
4.425
4.419
4.413
4.407
4.365
4.354
4.342
4.331
4.250
4.244
4.238
4.232
4.220
4.205
4.193
4.187
4.181
4.175
4.164
4.161
4.157
4.153
4.123
4.111
3.009
2.949
2.944
2.929
2.331
2.316
2.082
2.042
1.353
1.345
1.333
1.292
1.283
1.266
1.256
1.243
CO2Et
EtO2C
O
O
O
O
320
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm
1.00
1.00
1.01
0.99
1.04
1.04
2.18
2.16
1.09
1.02
1.05
1.11
3.66
2.82
3.37
3.85
4.60
2.00
171.505
165.853
136.480
131.449
130.160
128.805
109.714
108.242
80.710
78.289
77.282
77.070
76.906
76.859
69.213
61.496
60.709
53.878
40.409
38.607
34.852
28.093
26.504
25.356
25.114
14.219
14.187
14.168
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm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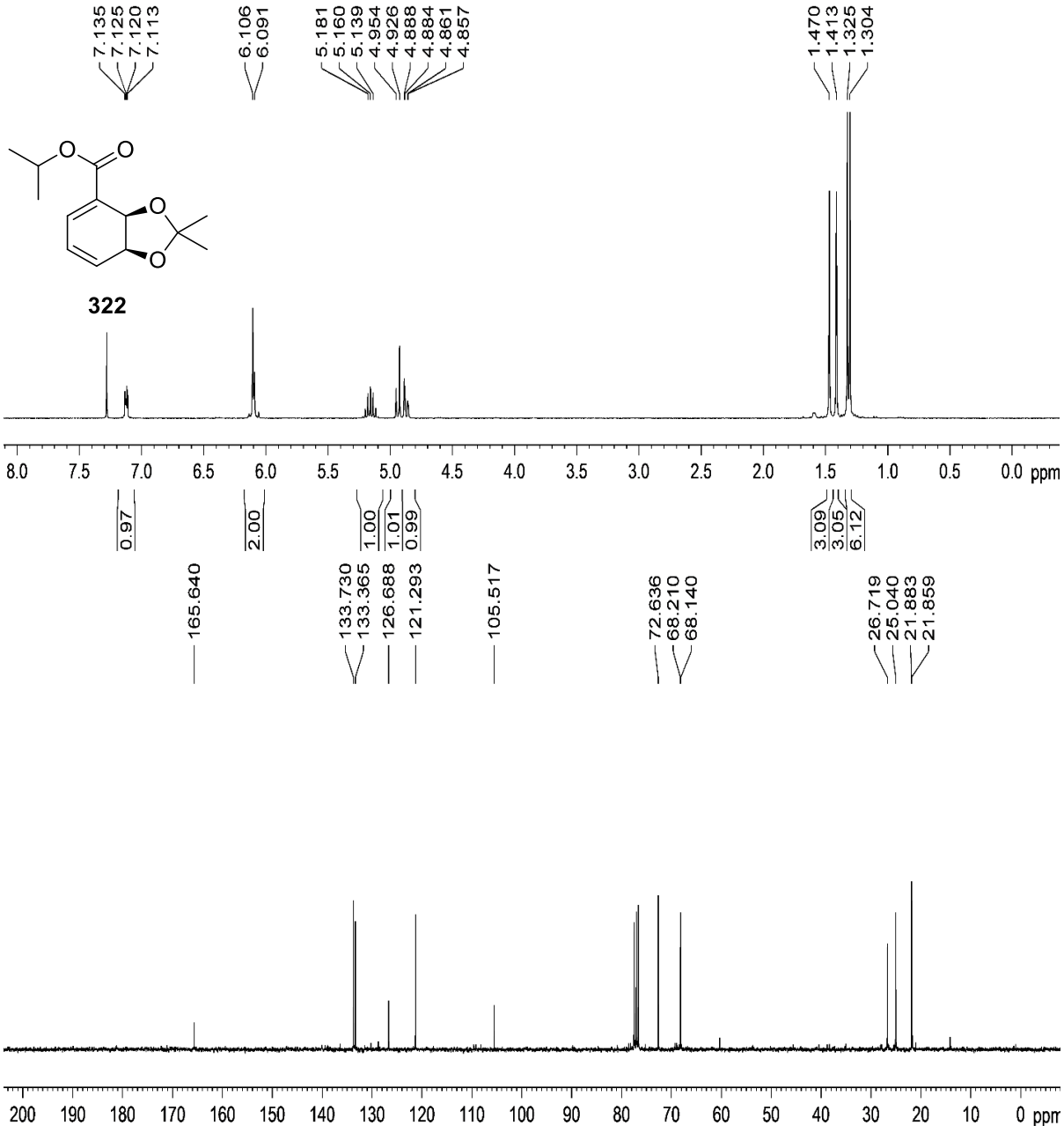
7.143
7.136
6.115
6.107
6.100
4.960
4.933
4.893
4.888
4.865
4.861
4.213
4.191
4.169
1.769
1.746
1.722
1.699
1.465
1.409
1.021
0.997
0.972
321
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm
0.95
2.00
0.98
0.97
2.04
2.11
0.02
3.05
3.02
3.16
166.210
133.786
133.612
126.399
121.332
105.551
72.590
68.161
66.393
26.728
25.028
22.016
10.451
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm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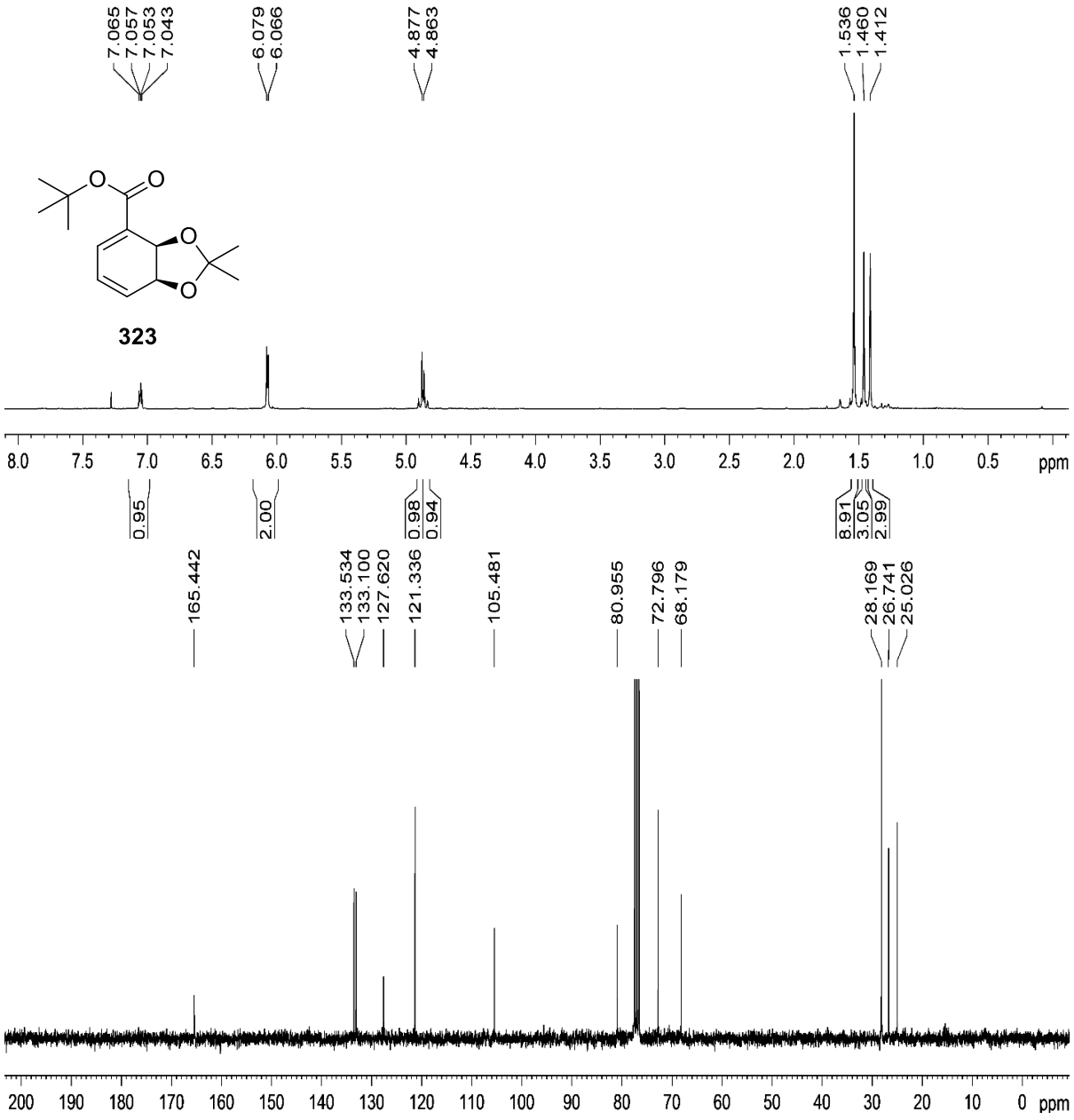
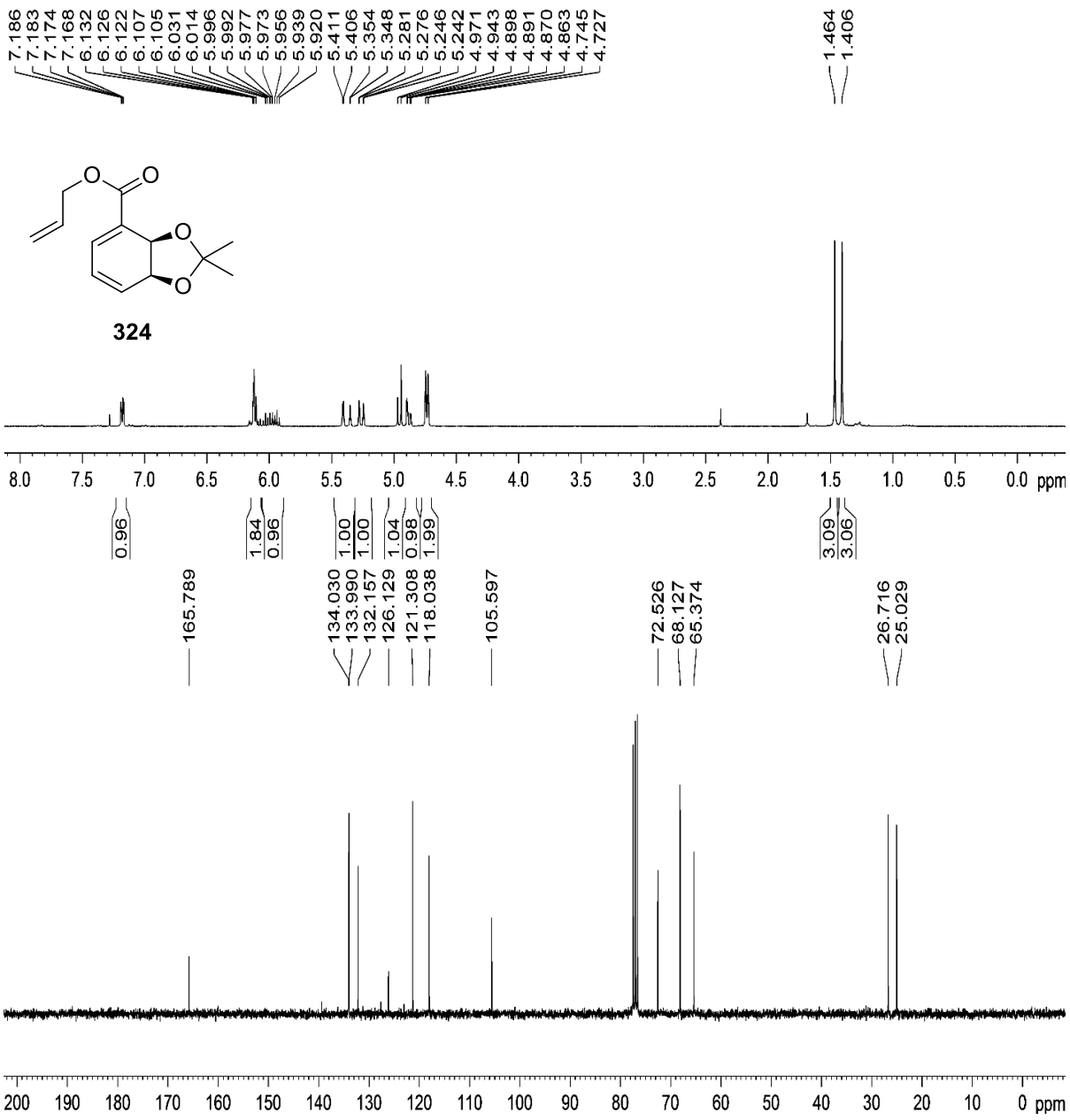
7.125
7.120
7.113
6.106
6.091
5.181
5.160
5.139
4.954
4.926
4.888
4.884
4.861
4.857
1.470
1.413
1.325
1.304
322
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm
0.97
2.00
1.00
1.01
0.99
3.09
3.05
6.12
165.640
133.730
133.365
126.688
121.293
105.517
72.636
68.210
68.140
26.719
25.040
21.883
21.859
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm


7.065
7.057
7.053
7.043
6.079
6.066
4.877
4.863
1.536
1.460
1.412
323
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
0.95
2.00
0.98
0.94
8.91
3.05
2.99
165.442
133.534
133.100
127.620
121.336
105.481
80.955
72.796
68.179
28.169
26.741
25.026
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm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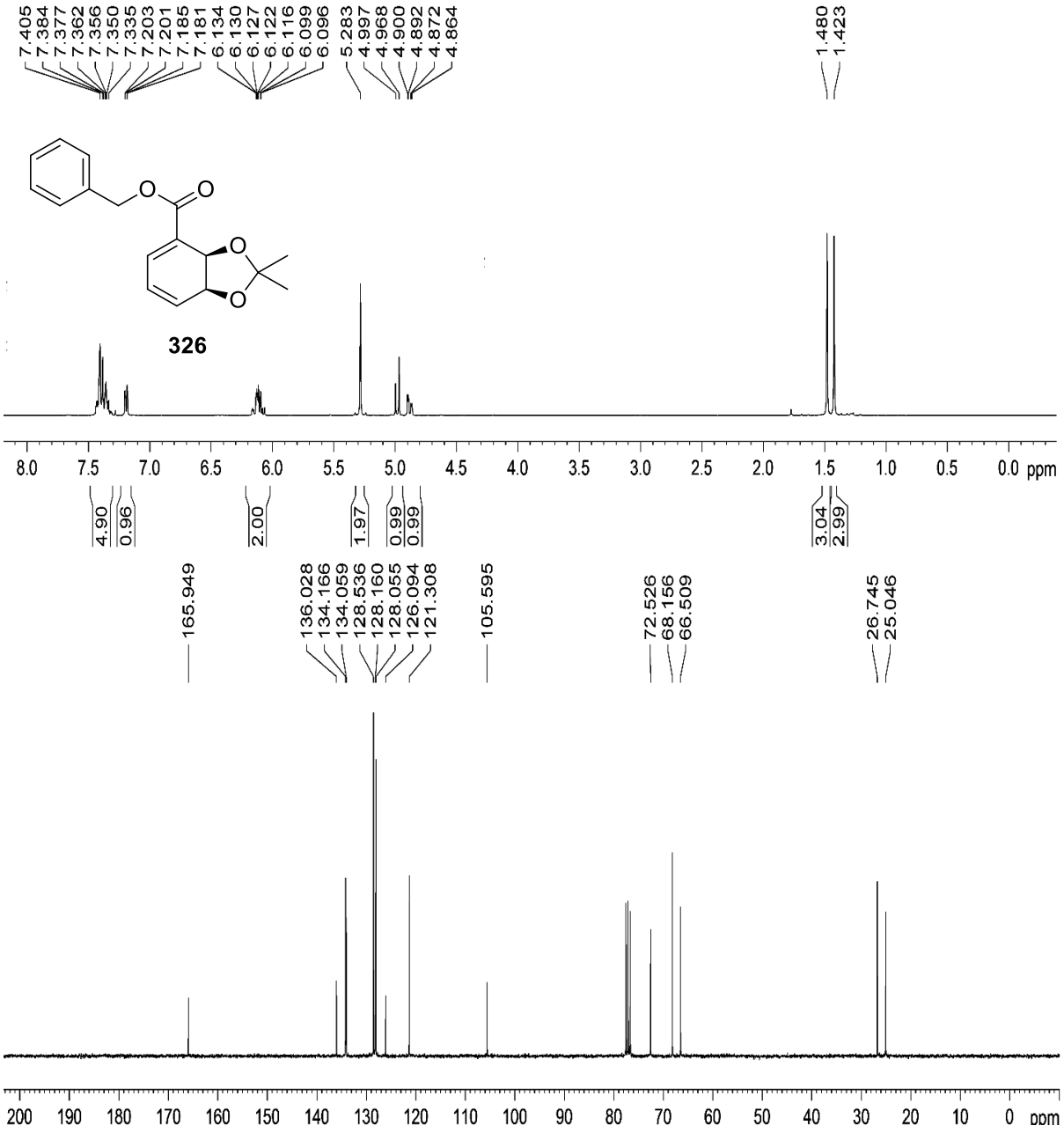
7.183
7.174
7.168
6.132
6.126
6.122
6.107
6.105
6.031
6.014
5.996
5.992
5.977
5.973
5.956
5.939
5.920
5.411
5.406
5.354
5.348
5.281
5.276
5.246
5.242
4.971
4.943
4.898
4.891
4.870
4.863
4.745
4.727
1.464
1.406
O
O
O
O
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm
0.96
1.84
0.96
1.00
1.00
1.04
0.98
1.99
3.09
3.06
165.789
134.030
133.990
132.157
126.129
121.308
118.038
105.597
72.526
68.127
65.374
26.716
25.029
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

324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7.384
7.377
7.362
7.356
7.350
7.335
7.203
7.201
7.185
7.181
6.134
6.130
6.127
6.122
6.116
6.099
6.096
5.283
4.997
4.968
4.900
4.892
4.872
4.864
1.480
1.423
326
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm
4.90
0.96
2.00
1.97
0.99
0.99
3.04
2.99
165.949
136.028
134.166
134.059
128.536
128.160
128.055
126.094
121.308
105.595
72.526
68.156
66.509
26.745
25.046
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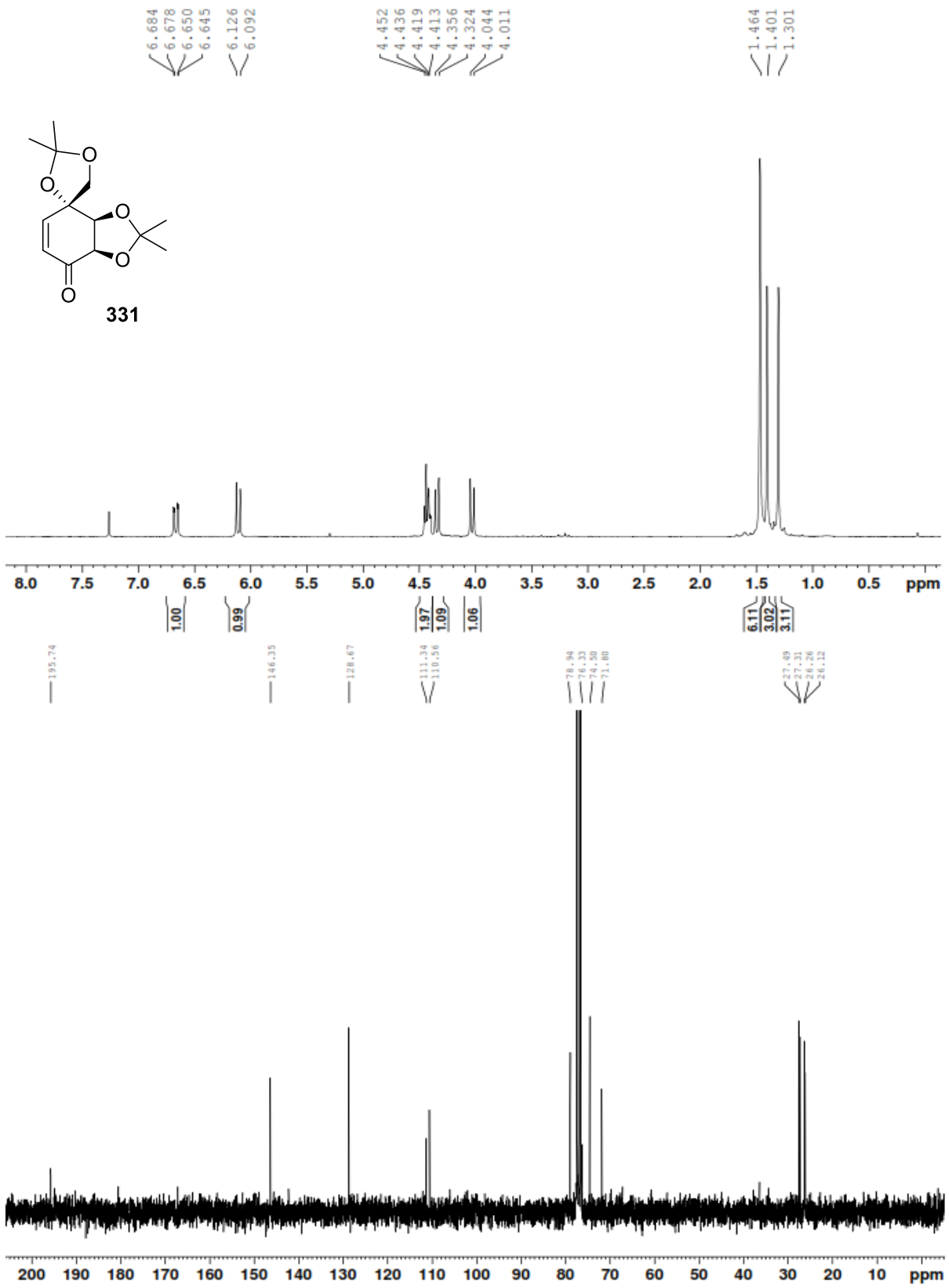
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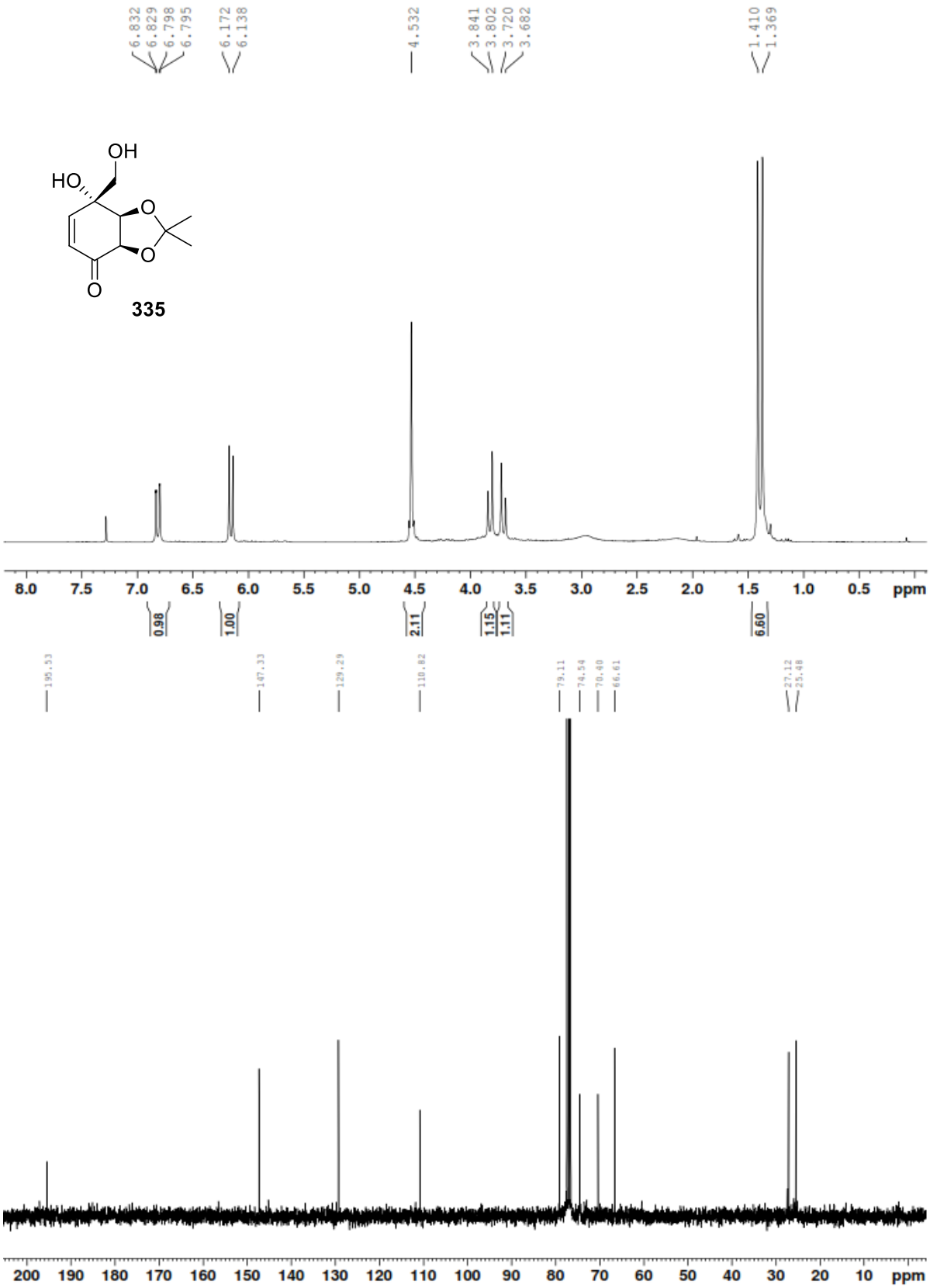
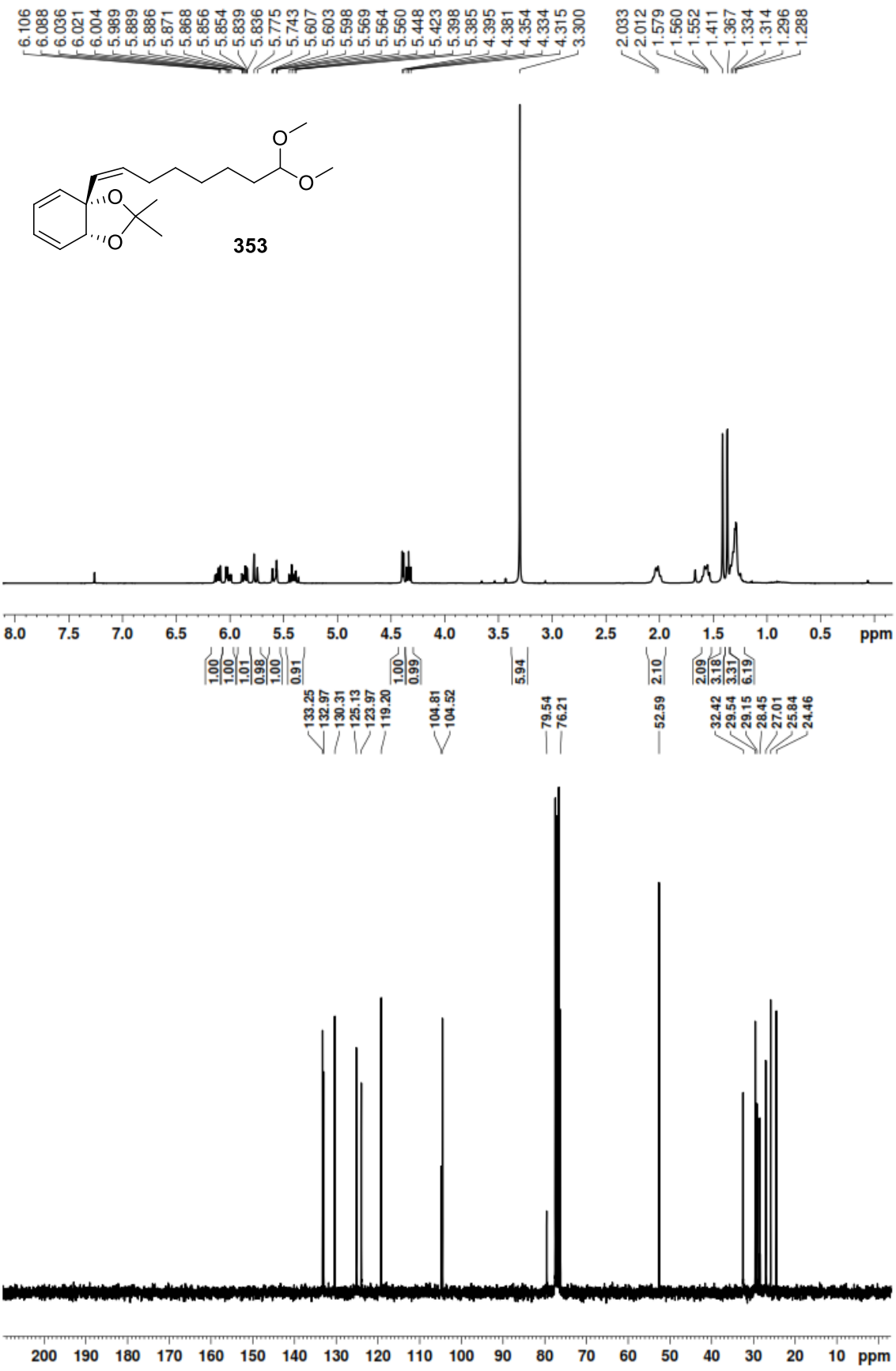
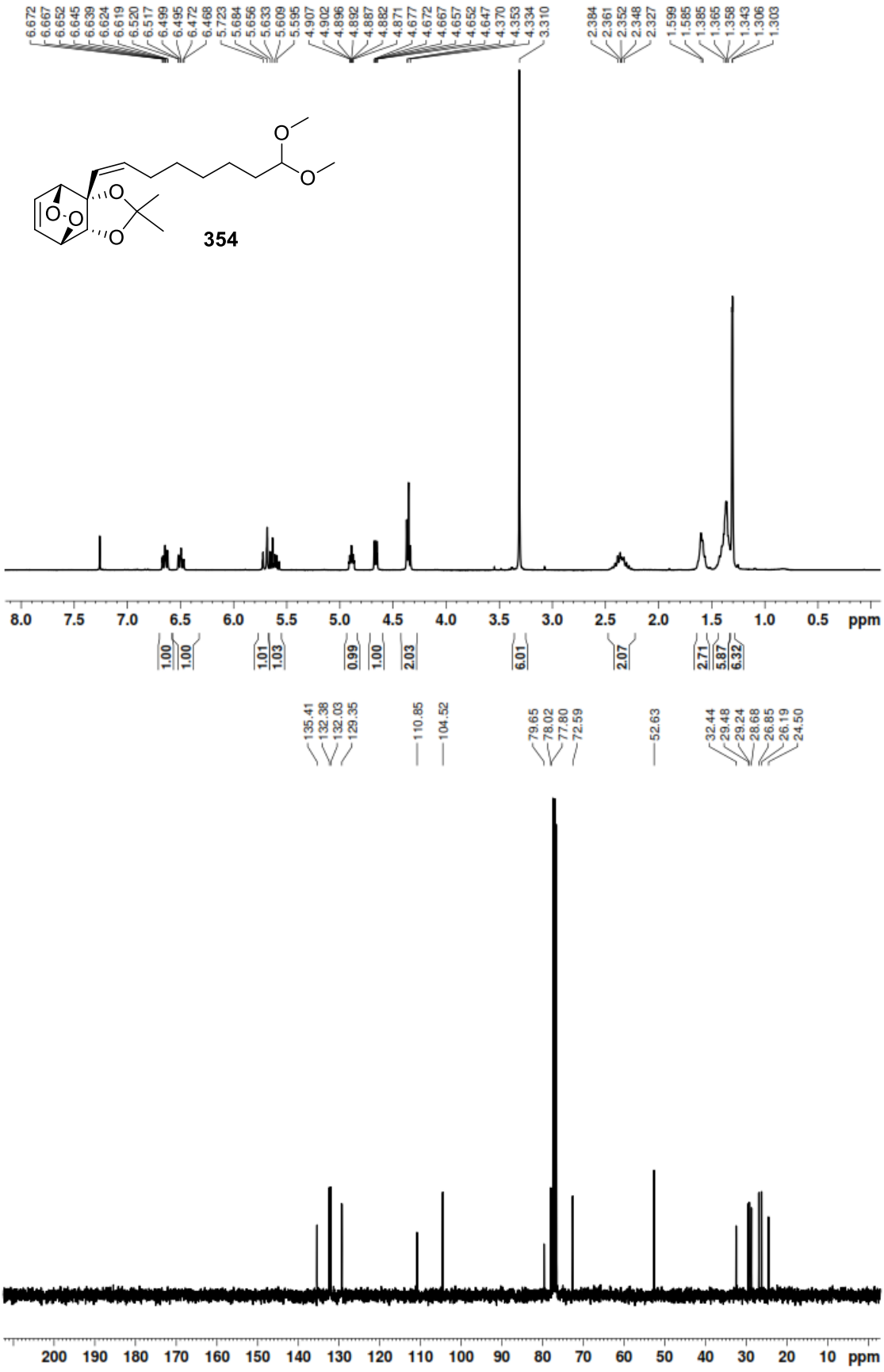
6.678
6.650
6.645
6.126
6.092
4.452
4.436
4.419
4.413
4.356
4.324
4.044
4.011
1.464
1.401
1.301
O
O
O
O
O
331
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
1.00
0.99
1.97
1.09
1.06
6.11
3.02
3.11
195.74
146.35
128.67
111.34
110.56
78.94
76.33
74.50
71.80
27.49
27.31
26.26
26.12
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm


6.832
6.829
6.798
6.795
6.172
6.138
4.532
3.841
3.802
3.720
3.682
1.410
1.369
OH
HO
O
O
O
335
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
0.98
1.00
2.11
1.15
1.11
6.60
195.53
147.33
129.29
110.82
79.11
74.54
70.40
66.61
27.12
25.48
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm


6.106
6.088
6.036
6.021
6.004
5.989
5.889
5.886
5.871
5.868
5.856
5.854
5.839
5.836
5.775
5.743
5.607
5.603
5.598
5.569
5.564
5.560
5.448
5.423
5.398
5.385
4.395
4.381
4.354
4.334
4.315
3.300
2.033
2.012
1.579
1.560
1.552
1.411
1.367
1.334
1.314
1.296
1.288
O
O
O
O
353
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
1.00 1.00 1.01 0.98 1.00 0.91 1.00 0.99 5.94 2.10 2.09 3.18 3.31 6.19
133.25 132.97 130.31 125.13 123.97 119.20 104.81 104.52 79.54 76.21 52.59 32.42 29.54 29.15 28.45 27.01 25.84 24.46
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm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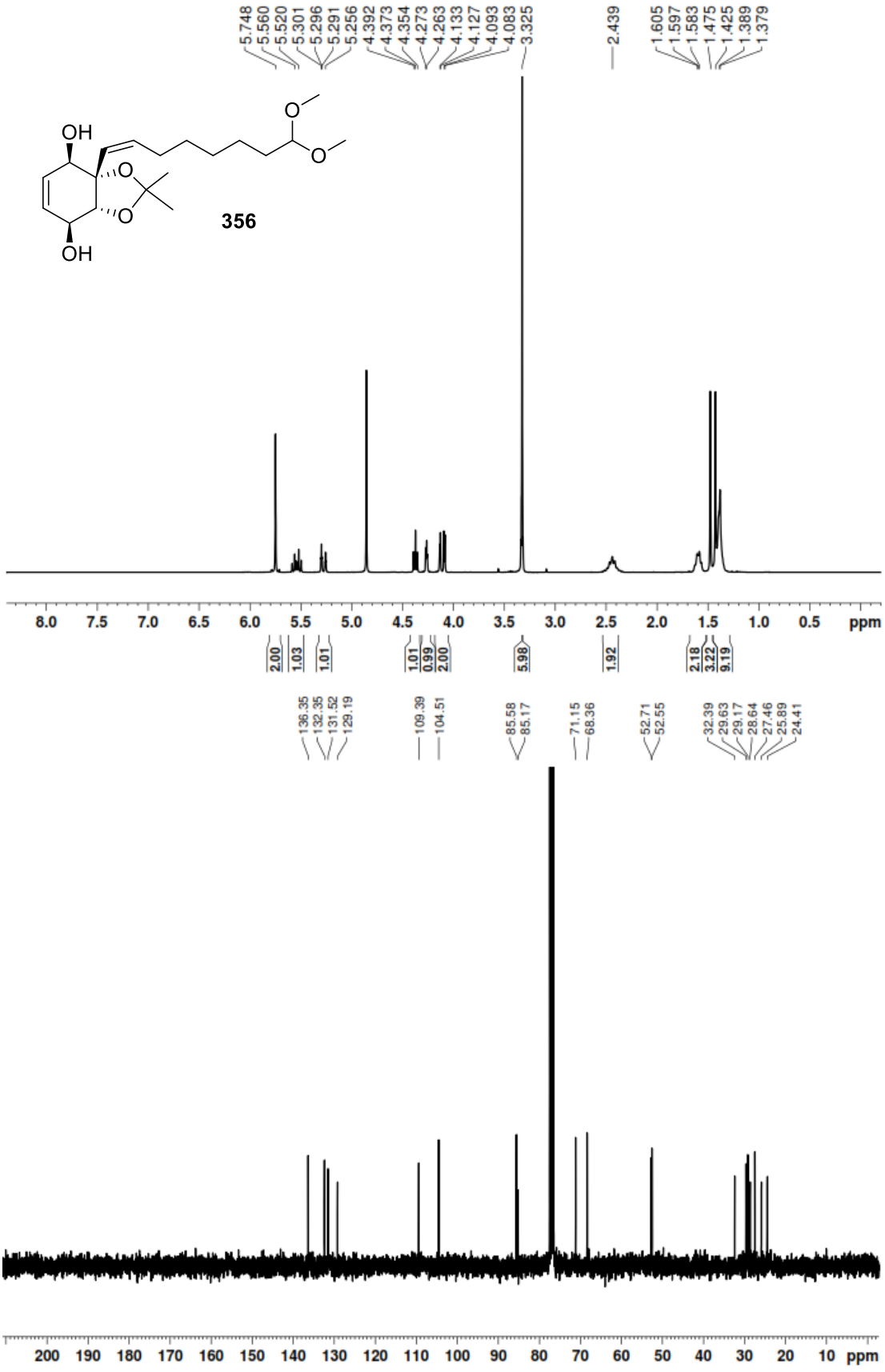
6.667
6.652
6.645
6.639
6.624
6.619
6.520
6.517
6.499
6.495
6.472
6.468
5.723
5.684
5.656
5.633
5.609
5.595
4.907
4.902
4.896
4.892
4.887
4.882
4.871
4.677
4.672
4.667
4.657
4.652
4.647
4.370
4.353
4.334
3.310
2.384
2.361
2.352
2.348
2.327
1.599
1.585
1.385
1.365
1.358
1.343
1.306
1.303
354
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
1.00 1.00 1.01 1.03 0.99 1.00 2.03 6.01 2.07 2.71 5.87 6.32
135.41
132.38
132.03
129.35
110.85
104.52
79.65
78.02
77.80
72.59
52.63
32.44
29.48
29.24
28.68
26.85
26.19
24.50
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm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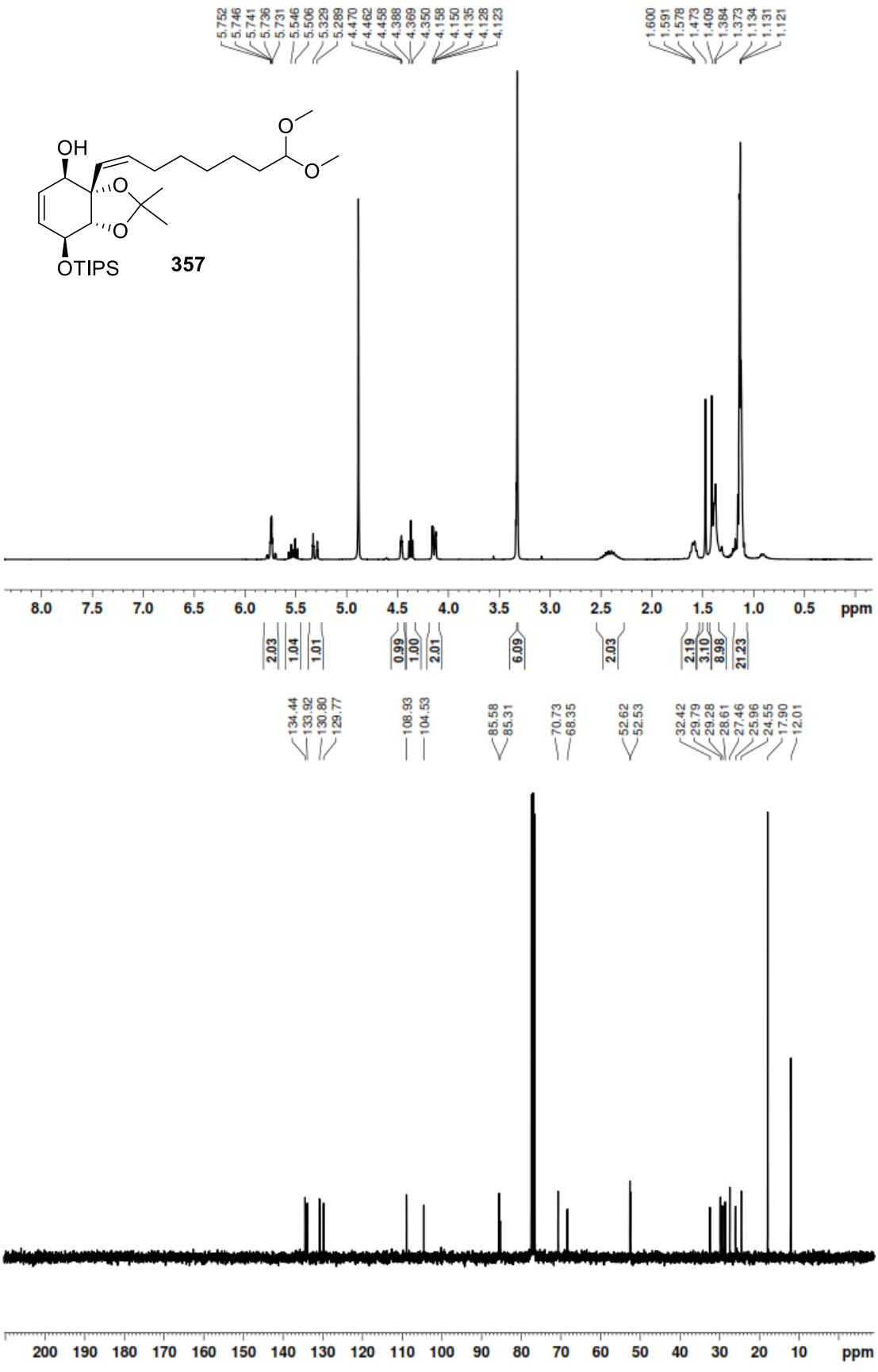
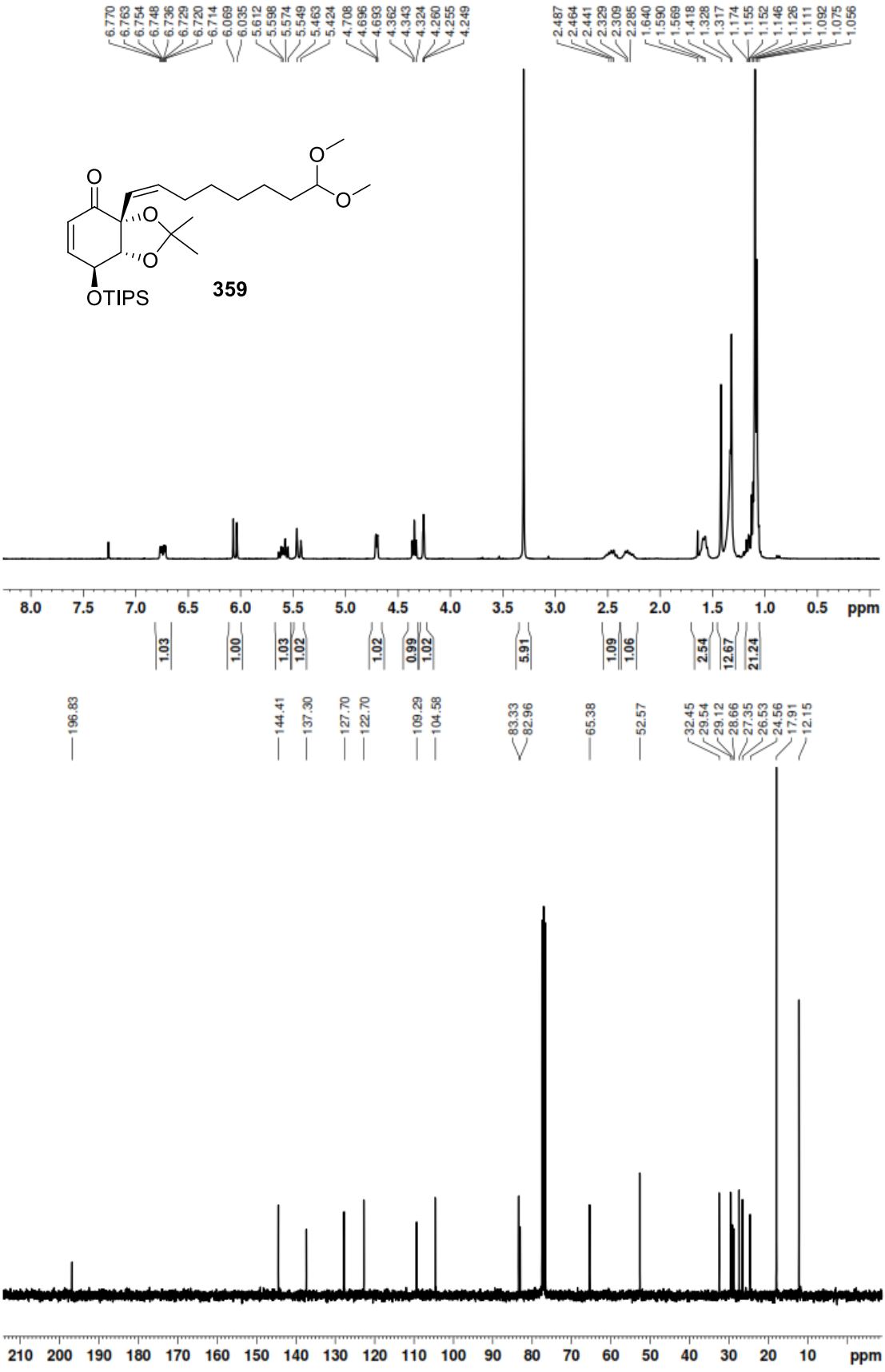
5.560
5.520
5.301
5.296
5.291
5.256
4.392
4.373
4.354
4.273
4.263
4.133
4.127
4.093
4.083
3.325
2.439
1.605
1.597
1.583
1.475
1.425
1.389
1.379
OH
O
O
O
O
OH
356
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
2.00
1.03
1.01
1.01
0.99
2.00
5.98
1.92
2.18
3.22
9.19
136.35
132.35
131.52
129.19
109.39
104.51
85.58
85.17
71.15
68.36
52.71
52.55
32.39
29.63
29.17
28.64
27.46
25.89
24.41
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm


OH
O
O
O
O
OTIPS
357
5.752
5.746
5.741
5.736
5.731
5.546
5.506
5.329
5.289
4.470
4.462
4.458
4.388
4.369
4.350
4.158
4.150
4.135
4.128
4.123
1.600
1.591
1.578
1.473
1.409
1.384
1.373
1.134
1.131
1.121
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
2.03
1.04
1.01
0.99
1.00
2.01
6.09
2.03
2.19
3.10
8.98
21.23
134.44
133.92
130.80
129.77
108.93
104.53
85.58
85.31
70.73
68.35
52.62
52.53
32.42
29.79
29.28
28.61
27.46
25.96
24.55
17.90
12.01
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm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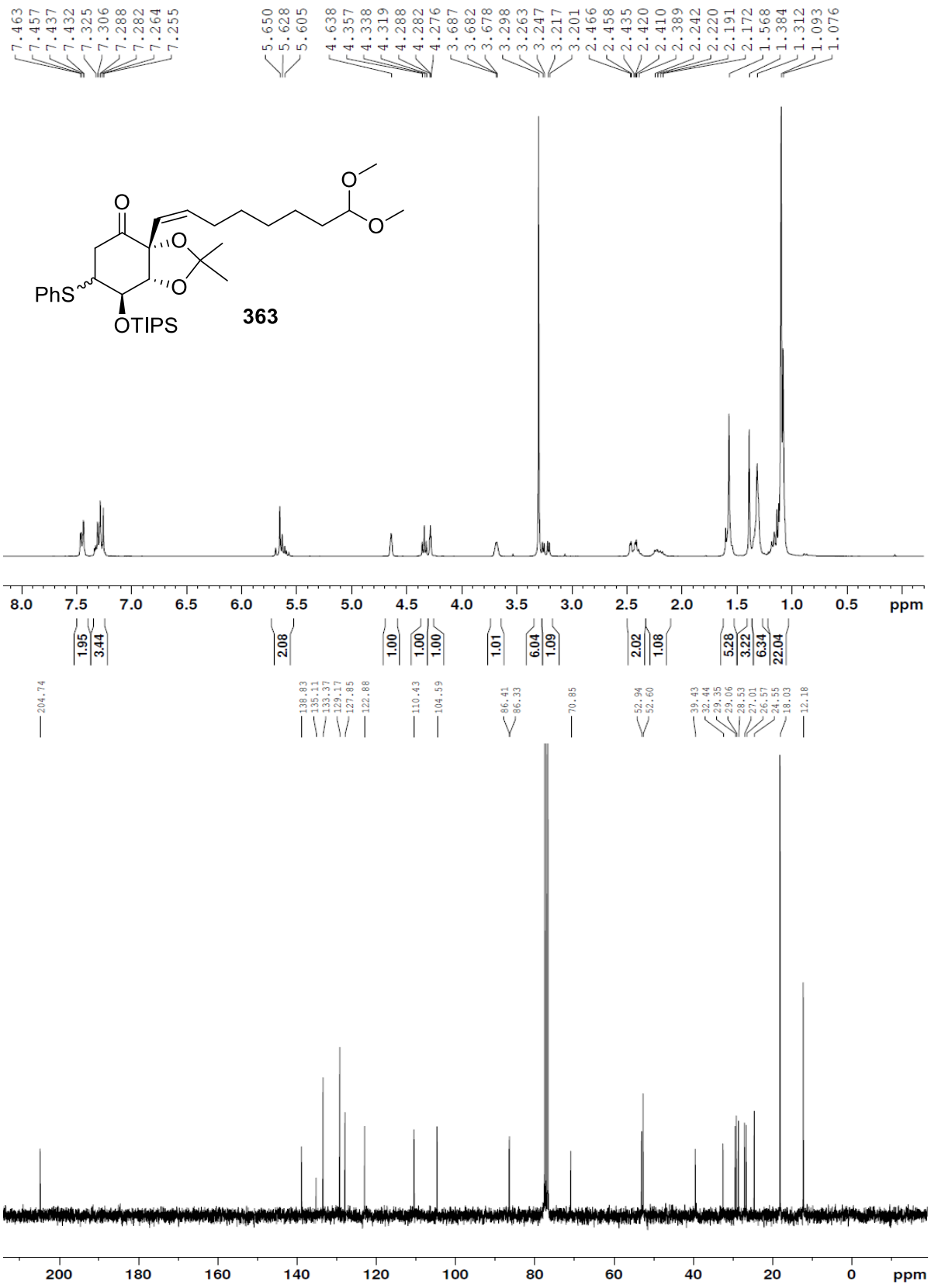
6.763
6.754
6.748
6.736
6.729
6.720
6.714
6.069
6.035
5.612
5.598
5.574
5.549
5.463
5.424
4.708
4.696
4.693
4.362
4.343
4.324
4.260
4.255
4.249
2.487
2.464
2.441
2.329
2.309
2.285
1.640
1.590
1.569
1.418
1.328
1.317
1.174
1.155
1.152
1.146
1.126
1.111
1.092
1.075
1.056
O
O
O
O
O
OTIPS
359
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
1.03
1.00
1.03
1.02
1.02
0.99
1.02
5.91
1.09
1.06
2.54
12.67
21.24
196.83
144.41
137.30
127.70
122.70
109.29
104.58
83.33
82.96
65.38
52.57
32.45
29.54
29.12
28.66
27.35
26.53
24.56
17.91
12.15
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm


7.463
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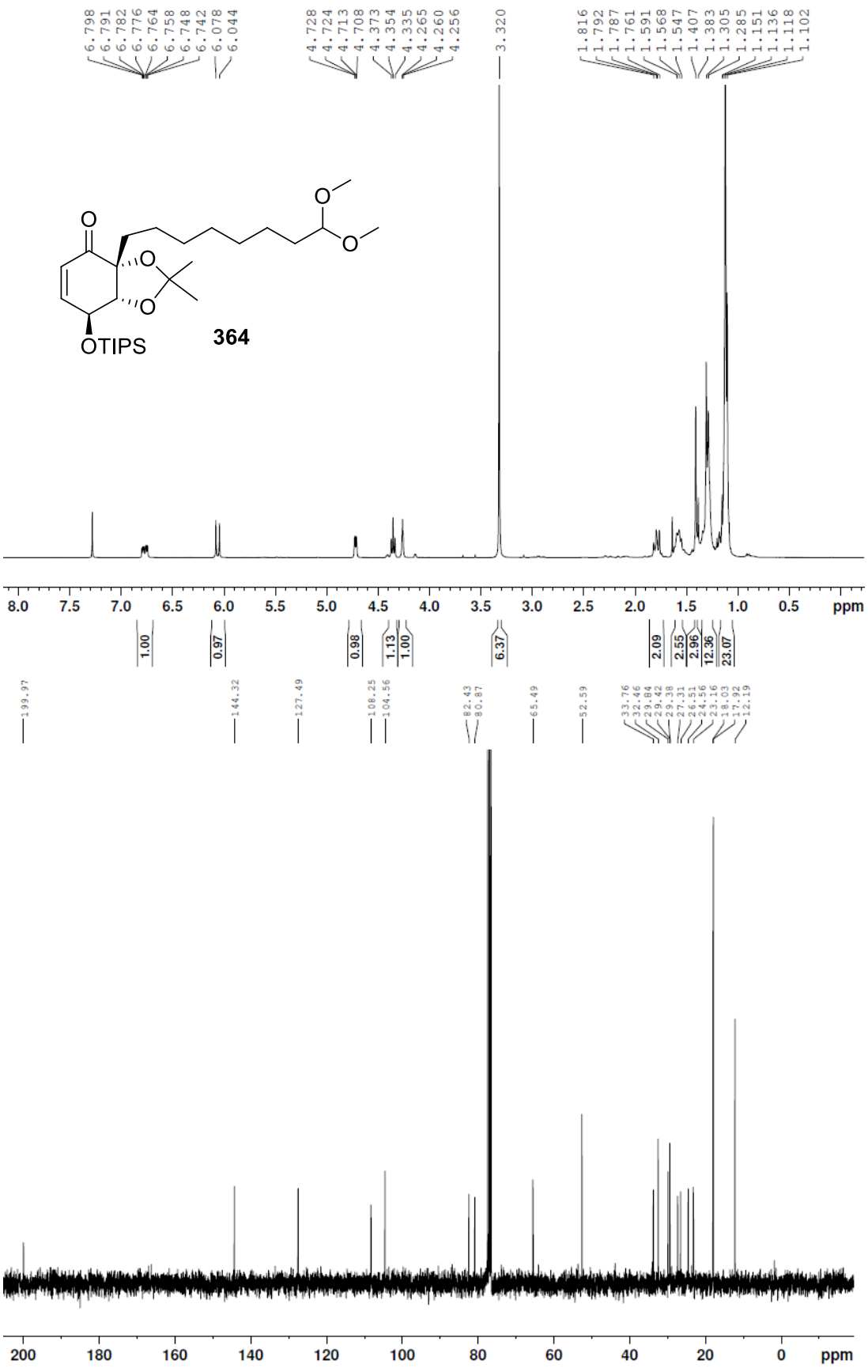
7.437
7.432
7.325
7.306
7.288
7.282
7.264
7.255
5.650
5.628
5.605
4.638
4.357
4.338
4.319
4.288
4.282
4.276
3.687
3.682
3.678
3.298
3.263
3.247
3.217
3.201
2.466
2.458
2.435
2.420
2.410
2.389
2.242
2.220
2.191
2.172
1.568
1.384
1.312
1.093
1.076
O
O
O
O
PhS
OTIPS
363
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
1.95
3.44
2.08
1.00
1.00
1.00
1.01
6.04
1.09
2.02
1.06
5.28
3.22
6.34
22.04
204.74
138.83
135.11
133.37
129.17
127.85
122.88
110.43
104.59
86.41
86.33
70.85
62.94
62.60
39.43
32.44
29.35
29.06
28.53
27.01
26.57
24.55
18.03
12.18
200
180
160
140
120
100
80
60
40
20
0
ppm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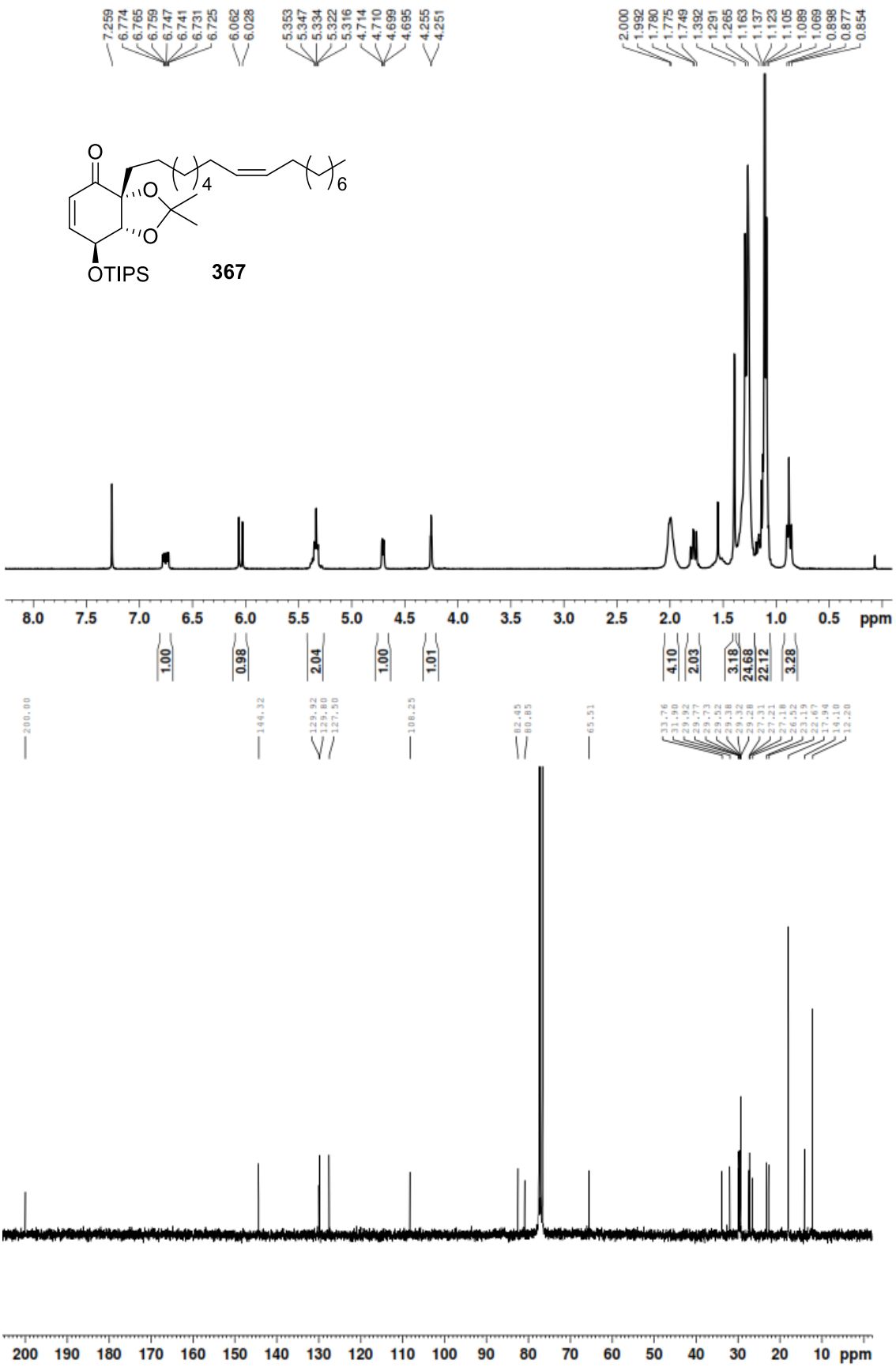
6.791
6.782
6.776
6.764
6.758
6.748
6.742
6.078
6.044
4.728
4.724
4.713
4.708
4.373
4.354
4.335
4.265
4.260
4.256
3.320
1.816
1.792
1.787
1.761
1.591
1.568
1.547
1.407
1.383
1.305
1.285
1.151
1.136
1.118
1.102
O
O
O
O
OTIPS
364
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
1.00
0.97
0.98
1.13
1.00
6.37
2.09
2.55
2.96
12.36
23.07
199.97
144.32
127.49
108.25
104.56
82.43
80.87
65.49
52.59
33.76
32.46
29.84
29.42
29.38
27.31
26.51
24.56
23.16
18.03
17.92
12.19
200
180
160
140
120
100
80
60
40
20
0
ppm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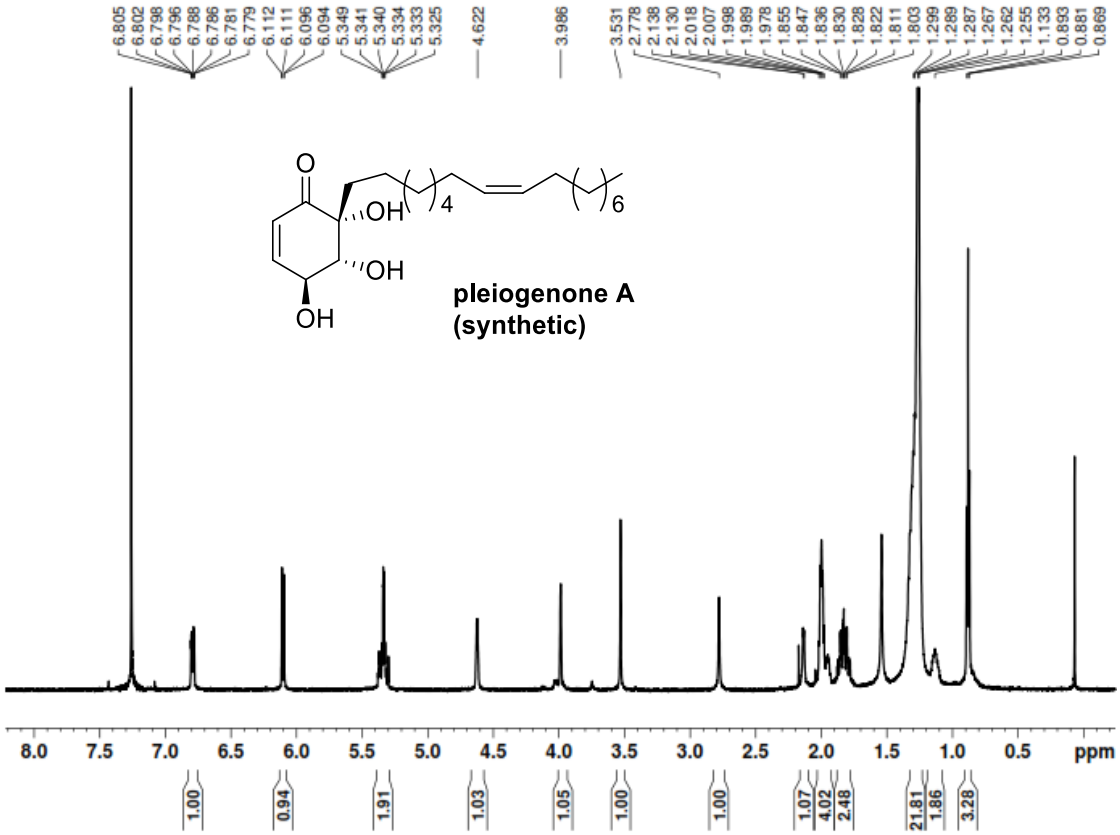
6.774
6.765
6.759
6.747
6.741
6.731
6.725
6.062
6.028
5.353
5.347
5.334
5.322
5.316
4.714
4.710
4.699
4.695
4.255
4.251
2.000
1.992
1.780
1.775
1.749
1.392
1.291
1.265
1.163
1.137
1.123
1.105
1.089
1.069
0.898
0.877
0.854
O
O
O
4
6
OTIPS
367
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
1.00
0.98
2.04
1.00
1.01
4.10
2.03
3.18
24.68
22.12
3.28
200.00
144.32
129.92
129.80
127.50
108.25
82.45
80.85
65.51
33.76
31.90
29.92
29.77
29.73
29.52
29.38
29.32
29.28
27.31
27.21
27.16
26.32
22.49
22.47
17.94
14.10
12.20
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm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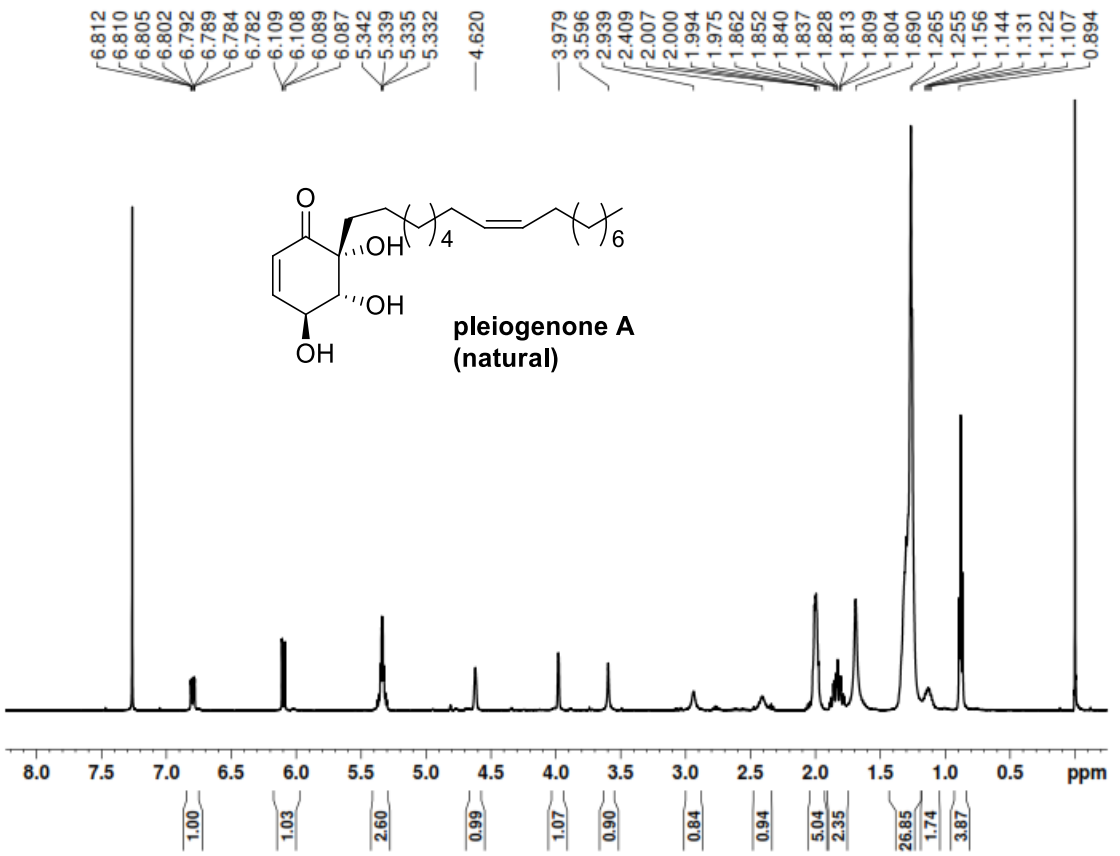
6.802
6.798
6.796
6.788
6.786
6.781
6.779
6.112
6.111
6.096
6.094
5.349
5.341
5.340
5.334
5.333
5.325
4.622
3.986
3.531
2.778
2.138
2.130
2.018
2.007
1.998
1.989
1.978
1.855
1.847
1.836
1.830
1.828
1.822
1.811
1.803
1.299
1.289
1.287
1.267
1.262
1.255
1.133
0.893
0.881
0.869
O
OH
OH
OH
4
6
pleiogenone A
(synthetic)
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
1.00
0.94
1.91
1.03
1.05
1.00
1.00
1.07
4.02
2.48
21.81
1.86
3.28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6.810
6.805
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6.792
6.789
6.784
6.782
6.109
6.108
6.089
6.087
5.342
5.339
5.335
5.332
4.620
3.979
3.596
2.939
2.409
2.007
2.000
1.994
1.975
1.862
1.852
1.840
1.837
1.828
1.813
1.809
1.804
1.690
1.265
1.255
1.156
1.144
1.131
1.122
1.107
0.894
O
OH
OH
OH
4
6
pleiogenone A
(natural)
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
1.00
1.03
2.60
0.99
1.07
0.90
0.84
0.94
5.04
2.35
26.85
1.74
3.87

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8. Vita

Jordan Froese was born on November 23, 1988 in Hamilton, Ontario, Canada to parents Thomas Froese and Kathi Crowley, and was raised jointly between southern Ontario and western New York. He was educated in Ontario, attending Lakeport Secondary School in St. Catharines. Jordan went on to pursue an Honours Bachelor of Science degree in biology and chemistry from the University of Toronto. Following the completion of his undergraduate work, he obtained a Bachelor of Education degree from Brock University in junior/intermediate education. Upon completing his education degree, he joined Professor Tomas Hudlicky's research group at Brock University as a Master's student, before transitioning to the doctoral program. Under the mentorship of Professor Hudlicky, Jordan has pursued research in enzymatic catalysis and natural product synthesis, and has become a life-long student of organic chemistry and biotechnology.